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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: 11/17/06

MEMORANDUM

SUBJECT: Chlormequat Chloride Toxicology Data Evaluation Records

PC Code: 018101
DP Barcode: D325193
TXR No.: 0054020

FROM: Karlyn J. Bailey, Toxicologist
Registration Action Branch 2
Health Effects Division (7509P)

THROUGH: Rick Loranger, Branch Senior Scientist
Registration Action Branch 2
Health Effects Division (7509P)

TO: Tony Kish, Risk Manager (RM22)
Fungicide Branch, Registration Division (7505P)

In the process of the toxicology review for a new use of chlormequat chloride, several new studies were reviewed. The MRIDS for the Data Evaluation Records are listed in Table 1.

Background: Chlormequat chloride [(2-chlorethyl) trimethylammonium chloride] is a plant growth regulator that belongs to the quaternary ammonium class of chemicals. Chlormequat chloride works through inhibition of gibberellin hormones, and the proposed use is on ornamental plants grown in containers in greenhouses, nurseries, and shadehouses.

Received in RRC
9/26/07

Table 1. List of Orthosulfamuron Toxicology Studies

Study Type		MRID #	Studies included in review	Comments
870.3100	Oral Subchronic Toxicity-Rat	00163408	N/A	New DER
870.3200	21-Day Dermal Toxicity -Rabbit	42246603	N/A	New DER
870.3700	Developmental Toxicity-Rat	42246604	N/A	New DER
870.3700	Developmental Toxicity-Rabbit	46715205	N/A	New DER
870.3800	2-Generation Reproduction	46715206	N/A	New DER
870.4100	Chronic Dog	46715201	N/A	New DER
870.5100	Bacterial Gene Mutation	41721610	N/A	New DER
870.5300	Mammalian Gene Mutation	41798102	N/A	New DER
870.5385	Mammalian Micronucleus	41798101	N/A	New DER
870.5550	Unscheduled DNA Synthesis	41798103	N/A	New DER

DATA EVALUATION RECORD

AC 38,555 (CHLORMEQUAT CHLORIDE)

Study Type: §82-1a, Subchronic Oral Toxicity Study in Rats

Work Assignment No. 3-01-74 B; formerly 2-01-74 B (MRID 00163408)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
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Prepared by
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** KJ
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 6/27/06
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** PVShah
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 7/12/06

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [feeding] - rat; OPPTS 870.3100a [§82-1a]; OECD 408.

PC CODE: 018101 **DP BARCODE:** D325193
TXR#: 0054020

TEST MATERIAL (PURITY): AC 38,555 (97.0% a.i.)

SYNONYMS: Chlormequat chloride; (2-chloroethyl) trimethyl ammonium chloride; Cycocel ®

CITATION: Tanabe, M. and Nagao, S. (1981) Three-month subacute dietary toxicity study of Cycocel ® in rats. Animal Research Corporation and Nibon University, Facility of Agriculture and Veterinary Medicine. Laboratory Project ID.: 523-JA-02-91, P-457. October 30, 1981. MRID 00163408. Unpublished.

SPONSOR: Not provided

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 00163408), Chlormequat chloride (AC 38,555; 97.0% (w/w) was administered for up to 13 weeks to 10 Sprague-Dawley rats/sex/group in the diet at dose levels of 0, 300, 900, or 2700 ppm (approximately equivalent to 0, 20.6, 61.3, and 188.5 in males and 0, 24.4, 72.9, and 220.1 mg/kg/day in females)

No adverse treatment-related effects were noted in mortality, clinical signs, food consumption, food efficiency, hematology, clinical chemistry, urinalysis, organ weights, or gross or microscopic pathology.

At 2700 ppm, there were decreases ($p \leq 0.05$) in body weight (7-8%; week 9-13), and overall body weight gain (11%; week 0-13) in males. No treatment-related effects on body weight were observed in females.

The LOAEL is 2700 ppm (approximately equivalent to 188.5 mg/kg/day in males and 220.1 mg/kg/day in females), based on decreased body weight and body weight gain in males. The NOAEL is 900 ppm (approximately equivalent to 61.3 in males and 72.9 mg/kg/day in females).

This study is classified as **acceptable/non-guideline** and does not satisfy the guideline requirement (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat based on insufficient data for the homogeneity and stability of the test material in the diet.

- **Note:** Homogeneity/stability was requested for this chemical on September 01, 2005. The registrant was unable to retrieve the data due to the time and place where the study was conducted (1981, Japan). A 4-week feeding study determining the stability of chlormequat in animal feed was submitted. The results showed that chlormequat chloride was homogenous and stable for up to 32 days at room temperature.

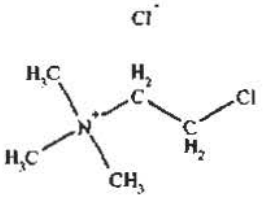
COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were not provided. However, the study was conducted prior to the adoption of GLP standards.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: AC 38,555

Description:	Not provided
Lot/Batch #:	Not provided
Purity:	97.0%
Compound Stability:	Not provided
CAS # of TGAI:	999-81-5
Structure:	



2. Vehicle - Diet, CE-2 powdered food

3. Test animals

Species:	Rat
Strain:	*CRJ:CD(SD)*
Age/weight at study initiation:	Approximately 5 weeks old; 128-145 g males, 105-125 g females
Source:	Japan Charles River Co., Ltd.
Housing:	Animals of the same sex were housed in pairs in stainless steel cages
Diet:	CE-2 powdered food (Japan Clea & Co. Inc.), 30 g for males and 20 g for females was offered daily
Water:	*ad libitum* (Source not provided)
Environmental conditions:	
Temperature:	22 ± 2°C
Humidity:	50-60%
Air changes:	Not reported
Photoperiod:	Not reported
Acclimation period:	One week

B. STUDY DESIGN

1. In life dates - Start: 01/14/81 End: Approximately 04/21/81

2. Animal assignment - Animals were assigned to the test groups shown in Table 1. The method of allocation was not stated.

Table 1. Study design [a]

Dose (ppm)	Mean Chemical Intake (mg/kg bw/day) [M/F]	# of Animals (M/F)
0	0/0	10/10
300	20.6/24.4	10/10
900	61.3/72.9	10/10
2700	188.5/220.1	10/10

a Data were obtained from the study report, pages 2 and 14.

3. **Dose selection rationale** - It was stated that doses for the current study were selected based on the results of a 3-week range-finding study. No further information was provided.

4. **Treatment preparation, administration, and analysis** - The appropriate amount of test substance was mixed with powdered feed. No further information was provided. Homogeneity and stability were not determined. Concentration of the dietary formulations was determined, but the dose levels and the times at which they were evaluated were unspecified.

Results - **Concentration (range as % nominal):** 90.4-101.7%

The provided information was insufficient to determine the adequacy of the mixing procedure or stability of the compound in the diet.

5. **Statistics** - Significant differences in body weight, food consumption, food efficiency, hematology, clinical chemistry, absolute and relative organ weights were determined by t-test. Significance was denoted at $p \leq 0.05$ or $p \leq 0.01$.

Multiple t-tests should not be performed without correcting for alpha-inflation by the Bonferroni or an alternative method. Tests for homogeneity of variance and normal distribution should have been performed to determine if the assumptions for analysis by parametric methods were appropriate.

C. METHODS

1. Observations

a. Cageside observations - Animals were checked for mortality and general health once daily.

b. Clinical examinations - Detailed clinical observations were not performed.

c. Neurological evaluations - No neurological evaluations were performed.

2. **Body weight** - All animals were weighed at initiation and weekly throughout the study. Overall (week 0-13) body weight gain was calculated at the end of the study.

3. **Food consumption and compound intake** - Weekly (g/rat/week) and total (g/rat) food consumption were reported. Weekly mean food efficiency (%) was calculated from body weight gain and food consumption data. Overall (Weeks 1-13) mean food efficiency was also reported. Compound intake (mg/kg/day) was calculated weekly, and mean compound intake (mg/kg/day) for the duration of the study was reported.

4. **Ophthalmoscopic examinations** - Ophthalmoscopic examinations were not performed.

5. **Hematology & clinical chemistry** - Blood for hematology and clinical chemistry evaluations was collected from all animals via the jugular vein following an overnight fast (approximately 15 hours) at Day 90. The following CHECKED (X) parameters were examined. Additionally, cholinesterase activity in the brain, plasma, and red blood cells was measured.

a. **Hematology**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
-	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
-	Platelet count*	X	Reticulocyte count
-	Blood clotting measurements*	X	Density
	(Activated partial thromboplastin time)		
	(Partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

\- Not examined

b. **Clinical chemistry**

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
-	Chloride	-	Creatinine*
-	Magnesium	X	Urea nitrogen*
-	Phosphorus	X	Total cholesterol*
X	Potassium*	-	Globulins
X	Sodium*	X	Glucose-fasting*

	ENZYMES		
X	Alkaline phosphatase (ALK)*	X	Total bilirubin
X	Cholinesterase (ChE) (Brain , plasma, red blood cells)	X	Total protein*
X	Creatine phosphokinase	-	Triglycerides
-	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin ratio
X	Alanine aminotransferase (ALT/also SGPT)*	-	Phospholipids
X	Aspartate aminotransferase (AST/also SGOT)*		
-	Sorbitol dehydrogenase*		
-	Gamma glutamyl transferase (GGT)*		
-	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

- Not examined

6. **Urinalysis** - Urine was collected from each animal monthly. The conditions of urine collection were not provided. The CHECKED (X) parameters were examined.

-	Appearance*	X	Glucose
-	Volume*	-	Ketones
-	Specific gravity*	-	Bilirubin
X	pH*	X	Blood*
-	Sediment (microscopic)	X	Urobilinogen
X	Protein*	-	Nitrite
-	Leukocytes		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

- Not examined

7. **Sacrifice and pathology** - Upon study termination, all animals were sacrificed and subjected to gross pathological examination. It was stated that the CHECKED (X) tissues were examined microscopically. However, only findings in the heart, lung, liver, kidney, spleen, sciatic nerve, and testes were presented. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
-	Tongue	-	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord*
X	Stomach*	-	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes*

X	Jejunum*	XX	Thymus*+			**GLANDULAR**
X	Ileum*				XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**		-	Lacrimal gland
X	Colon*	XX	Kidneys*+		-	Parathyroids*
X	Rectum*	X	Urinary bladder*		XX	Thyroid*
XX	Liver*+	XX	Testes*+			**OTHER**
-	Gall bladder (not rat)*	-	Epididymides*+		-	Bone (*femur and/or sternum*)
-	Bile duct (rat)	-	Prostate*		-	Femur (including knee joint)
X	Pancreas*	X	*Seminal vesicles**		X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+		X	Skin*
X	Trachea*	X	Uterus*+			All gross lesions and masses*
XX	Lung*	-	Mammary gland*			
-	Nose*	-	Vagina			
-	Pharynx*					
-	Larynx*					

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.
\- Not examined

II. RESULTS

A. OBSERVATIONS

1. ***Clinical signs of toxicity*** - No abnormality was listed for any animal at any time throughout the study.

2. **Mortality** - All animals survived to the end of the study.

B. BODY WEIGHT AND WEIGHT GAIN - Body weight in the 2700 ppm males was decreased (7-8%; $p \leq 0.05$) in Weeks 9-13 (Table 2). Overall (Week 0-13) body weight gain was also decreased (11%; $p \leq 0.05$) in these animals. No treatment-related effects on body weight or body weight gain were observed in the males at 300 or 900 ppm or in the females at any dose.

Table 2. Mean (±SD) body weights and body weight gains (g) in rats treated with AC 38,555 in the diet for 13 weeks.[a]

Study Week	Dose (ppm)			
	0	300	900	2700
	Males			
0	135±6	137±5	135±4	136±5
1	199±6	200±7	200±7	198±8
9	442±27	432±24	427±17	411±23* (↓7)
11	475±33	461±21	454±18	438±27* (↓8)
13	503±34	494±20	495±28	465±31* (↓8)

Study Week	Dose (ppm)			
	0	300	900	2700
	Males			
Overall (0-13) gain	368±36	357±20	360±29	329±31* (↓11)
	Females			
0	116±4	115±4	116±4	116±5
1	162±6	160±8	159±4	163±5
13	304±16	295±19	297±18	297±18
Overall (0-13) gain	188±16	180±17	181±16	181±19

a Data were obtained from Table 1 on page 12; n=10. Data presented parenthetically represent percent difference from controls (calculated by reviewers)

* Significantly different from controls at p≤0.05

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

1. Food consumption - Minor decreases (3-6%; p≤0.01) in food consumption were noted in the 2700 ppm males in Weeks 10-13 (Table 3). However, overall (Week 0-13) food consumption in males at this dose was similar to controls. No treatment-related effects on food consumption were observed in the males at 300 or 900 ppm or in the females at any dose.

Table 3. Mean (±SD) food consumption (g/rat/week) in male rats treated with AC 38,555 in the diet for 13 weeks. [a]

Study Week	Dose (ppm)			
	0	300	900	2700
0	152±6	155±5	147±11	152±9
10	174±1	173±1	173±2	168±2**(↓3)
11	174±3	174±1	173±2	167±1**(↓4)
12	176±1	174±1	173±1	166±1**(↓6)
13	176±1	175±1	174±1	166±1**(↓6)
Overall (0-13)	2226	2236	2229	2200

a Data were obtained from Table 2 on page 13; n=10. Data presented parenthetically represent percent difference from controls (calculated by reviewers)

** Significantly different from controls at p≤0.01

2. Food efficiency -No treatment-related effect was observed on food efficiency. Only transient differences (p≤0.05) were observed. These differences were often not dose-related, and overall (Weeks 1-13) mean food efficiency in both sexes was similar to controls.

3. Compound consumption - Group mean chemical intake values (mg/kg/day) are reported in Table 1.

D. BLOOD ANALYSES

1. Hematology - No treatment-related effects on hematology were observed. All significant

($p \leq 0.05$) differences in hematology were unrelated to dose and/or the magnitude was minor and not considered biologically significant.

2. **Clinical chemistry** – There were no treatment-related effects observed on clinical chemistry. Brain cholinesterase activity was decreased (19%; $p \leq 0.05$) in the 2700 ppm males, and red blood cell cholinesterase activity was increased (33-88%; $p \leq 0.05$) in the 900 ppm males and in the $\geq$ 900 ppm females (Table 4). However, these differences were not considered toxicologically significant.

Table 4. Mean (±SD) cholinesterase activity in rats treated with AC 38,555 in the diet for 13 weeks. [a]

Study Week	Dose (ppm)			
	0	300	900	2700
Males				
Brain (μmol/g)	73.0±15.1	77.6±19.0	68.9±17.5	58.9±13.8*(↓19)
Plasma (μmol/0.1ml)	5.13±2.10	4.18±1.69	4.21±1.70	5.86±1.85
Red blood cell (ΔPH/hr)	0.15±0.06	0.18±0.03	0.20±0.04*(↑33)	0.19±0.03(↑27)
Females				
Brain (μmol/g)	59.2±10.5	54.2±17.0	74.6±23.9	57.4±29.3
Plasma (μmol/0.1ml)	22.37±8.40	21.27±5.69	19.97±5.14	16.73±6.80
Red blood cell (ΔPH/hr)	0.08±0.02	0.09±0.02	0.13±0.02**(↑63)	0.15±0.05**(↑88)

a Data were obtained from Table 6-2 on page 18 of the study report; n=10. Data presented parenthetically represent percent difference from controls (calculated by reviewers)

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

E. URINALYSIS - No treatment-related effects on urinalysis were observed.

F. SACRIFICE AND PATHOLOGY

1. **Organ weight** - Terminal body weights were decreased (7%; $p \leq 0.05$) in the 2700 ppm males. There were no treatment-related effects on absolute or relative to body organ weights.

2. **Gross pathology** - No treatment-related gross lesions were noted at any dose.

3. **Microscopic pathology** - No treatment-related microscopic lesions were observed.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigators concluded that the LOAEL was 2700 ppm based on decreased body weight, body weight gain, and food consumption in males. The NOAEL was 900 ppm.

B. REVIEWER COMMENTS - No adverse treatment-related effects were noted in mortality,

clinical signs, hematology, clinical chemistry, cholinesterase activity, urinalysis, organ weights, or gross or microscopic pathology.

At 2700 ppm, there were decreases ($p \leq 0.05$) in body weight (7-8%; week 9-13), and overall body weight gain (11%; week 0-13) in males. No treatment-related effects on body weight were observed in females. Minor decreases ($p \leq 0.01$) in food consumption were noted.

The LOAEL is 2700 ppm (approximately equivalent to 188.5 mg/kg/day in males and 220.1 mg/kg/day in females), based on decreased body weight and body weight gain in males. The NOAEL is 900 ppm (approximately equivalent to 61.3 in males and 72.9 mg/kg/day in females).

This study is classified as **acceptable/non-guideline** and does not satisfy the guideline requirement (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat based on insufficient data for the homogeneity and stability of the test compound in the diet.

C. **STUDY DEFICIENCIES** - This study was performed prior to the adoption of the Pesticide Assessment Guidelines (Subdivision F, 1984). However, irrespective of the date the study was conducted, the following deficiencies were observed:

- Homogeneity and stability of the test compound in the diet were not reported.
- Conditions of urine collection were not described.
- Multiple t-tests should not have been performed without correcting for alpha-inflation by the Bonferroni or an alternative method. Analysis of variance should have been performed prior to pairwise comparisons.

- **Note:** Homogeneity/stability was requested for this chemical on September 01, 2005. Unfortunately, the registrant was unable to retrieve the data due to the time and place where the study was conducted (1981, Japan). A 4-week feeding study determining the stability of chlormequat in animal feed was submitted. The results showed that chlormequat chloride was homogenous and stable for up to 32 days at room temperature.

DATA FOR ENTRY INTO ISIS

90-Day Study - rats (870.3100a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg	Doses tested mg/kg	NOAEL mg/kg	LOAEL mg/kg	Endpoints	Comments
109709	00163408	subchronic	rat	90 days	oral	diet	20.6-220.1	0/0, 20.6/24.4, 61.3/72.9, and 188.5/220.1 [M/F]	61.3	188.5	BW, BWG	

DATA EVALUATION REPORT

AC 38,555 (CLORMEQUAT CHLORIDE)

Study Type: §82-2; 21-Day Dermal Toxicity Study - Rabbits

Work Assignment No. 2-01-74 C (MRID 42246603)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
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Date: 06-27-05

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Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/30/05

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T

Signature: Steven Brecher
Date: 6/28/05

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** _____
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 6/27/06
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** _____
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 7/12/06

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Subchronic 21-Day Dermal Toxicity - Rabbit; OPPTS 870.3200 [§82-2]; OECD 410.

PC CODE: 018101 **DP BARCODE:** D325193
TXR #: 0054020

TEST MATERIAL (PURITY): Clormequat chloride (AC 38,555; 66.1% a.i., doses adjusted for purity)

SYNONYMS: (2-Chloroethyl) trimethylammonium chloride

CITATION: Goad, M.E.P. (1991) Twenty-one day dermal toxicity study with AC 38,555 in rabbits. Arthur D. Little, Inc., Cambridge, MA. Laboratory Project ID.: 67745-00, November 4, 1991. MRID 42246603. Unpublished.

SPONSOR: American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study (MRID 42246603), Chlormequat chloride (AC 38,555; 66.1% a.i.; Lot #: AC 6779-98A) was applied to the shaved intact skin of 6 New Zealand White rabbits/sex/dose at dose levels of 0, 115, 345, or 1035 mg/kg/day (> the limit dose), 6 hours/day, 5 days/week for 3 weeks (15 applications). Doses were adjusted for purity. Dermal irritation was evaluated daily using a Draize-like method. Because the initial controls were inadvertently exposed to the test material (1035 mg/kg) for 2.5 hours on Day 1, additional control animals (Group 5) were added to the study on Day 2 to provide gross necropsy and histopathology data.

No compound-related effects on mortality, clinical signs, body weight, body weight gain, food consumption, hematology, clinical chemistry, or organ weights were observed.

In the 345 mg/kg/day group, dermal irritation characterized by erythema, edema, and fissuring was observed in 1-2 females, a gross lesion (foci) was seen in one female, and histopathological lesions in the treated skin (mild acanthosis and minimal subacute inflammation and edema) in another female.

At 1035 mg/kg/day, dermal irritation (erythema, edema, fissuring, and eschar) was also observed in 1-3 females. Gross lesions were limited to foci in the treated skin in 2 males and 1 female. Minimal to mild histopathological effects observed in the treated skin in both sexes included: (i) acanthosis (2 males and 4 females); (ii) subacute inflammation (2 males and 1 female); and (iii) edema (2 males and 3 females). Additionally, a single male displayed minimal fibrosis of the treated skin.

The systemic LOAEL is not determined and the systemic NOAEL is 1035 mg/kg/day (> the limit dose).

The dermal LOAEL is 345 mg/kg/day based on dermal irritation, gross lesion of the treated skin, acanthosis, subacute inflammation, and edema in females. The dermal NOAEL is 115 mg/kg/day.

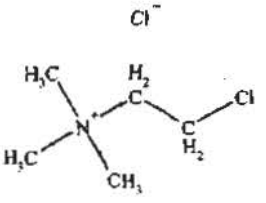
This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3200; OECD 410) for a 21-day dermal toxicity study in rabbits.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** AC 38,555 (Clormequat chloride)

Description:	Not reported
Lot #:	AC 6779-98A
Purity:	66.1% a.i. (doses adjusted for purity)
Compound stability:	Not reported
CAS # of TGAI:	999-81-5
Structure:	

2. **Vehicle** - Distilled water
3. **Test animals**

Species:	Rabbit
Strain:	New Zealand White
Age/weight at Day 1:	Approximately 12-14 weeks/ 1.9-2.5 kg males and 1.9-2.3 kg females (Groups 1-4) 3.3-3.7 kg males and 3.5-3.8 kg females (Group 5)
Source:	Hazleton Research Products, Inc. (Denver, PA)
Housing:	Individually in suspended stainless steel cages with wire mesh floors
Diet:	Purina certified high fiber rabbit chow (#5325), *ad libitum*, except for overnight fasting prior to sacrifice
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 16-25°C 45-67% **Humidity:** Not reported **Air changes:** 12 hrs dark/12 hrs light **Photoperiod:**
Acclimation period:	≥13 days

B. STUDY DESIGN

1. **In-life dates** - Start: 09/03/91 End: 10/02/91

2. **Animal assignment** - Animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1.

Table 1. Study design [a]

Groups		Dose (mg/kg bw)	# of Males	# of Females
Group 1	Control	0	6	6
Group 2	Low	115	6	6
Group 3	Mid	345	6	6
Group 4	High	1035	6	6
Group 5 [b]	Control	0	6	6

a Data were obtained from pages 11-12 of the study report.

b Because the Group 1 controls were inadvertently exposed to the test material for 2.5 hours on Day 1, additional control animals (Group 5) were added to the study on Day 2 to provide gross necropsy and histopathology data. However, as the Group 5 animals were older and they weighed more, the body weights, food consumption, organ weights, and clinical pathology data were not compared to the treated groups.

3. **Dose-selection rationale** - The doses presented in Table 1 were based on the results of a range-finding study, in which animals were exposed dermally to the test material at doses of 345, 460, 575, 862.5, 977.5, or 1150 mg/kg for 1 to 7 consecutive days. At 1150 mg/kg, one rabbit died within 4 hours of treatment. All other animals survived to scheduled sacrifice. No further information was provided.

4. **Preparation and treatment of animal skin** - Approximately 24 hours before the first application and approximately weekly thereafter, the fur was clipped from the dorsal area of the trunk of each animal (at least 10% of the total body surface area). The test sites were not abraded. The applied quantities of the test substance were adjusted weekly according to individual animal body weights. The test substance was applied to a porous 8-ply gauze dressing which was then applied to the test site, and held in place with non-occlusive surgical tape. The dressings were removed after 6 hours, and excess test material was washed from the skin with water. Rabbits in the control group were exposed to distilled water using the same procedure as described for the treated animals.

5. **Statistics** - The data were subjected to the following statistical analyses:

Parameter	Statistical Tests
Food consumption and body weight	Repeated measures ANOVA followed by Duncan's multiple comparisons test, as necessary
Hematology, clinical chemistry, and organ weight	One-way ANOVA followed by Duncan's multiple comparisons test, as necessary

It was not reported if the data were checked for normality and homogeneity of variance. These assumptions should be met before proceeding with parametric analyses. Otherwise, the statistical methods were considered appropriate. Significance was denoted at $p \leq 0.05$ and $p \leq 0.01$.

C. METHODS

1. Observations

a. Cageside observations - Animals were observed daily for mortality, moribundity, and clinical signs of toxicity.

b. Clinical examinations - All animals were examined daily at the conclusion of treatment for signs of local skin irritation. Skin irritation was graded using the scale provided in Appendix C on page 68 of the study report.

c. Neurological evaluations - Neurological evaluations were not performed.

2. Body weight - Animals were weighed at randomization, on Days 1 (prior to dosing), 8, 15, and 22 (termination).

3. Food consumption - Food consumption was measured weekly for each animal at intervals Days 1-8, 8-15, and 15-21. Absolute and relative food consumption were reported as g food/animal/week and g food/kg bw/week, respectively. Food efficiency was not reported.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were not performed.

5. Hematology and clinical chemistry - Prior to initiation of dosing, blood was collected via an artery or vein of the ear from each rabbit (non-fasted), under acepromazone (i.v.) anesthesia. At termination, blood was collected via cardiac puncture from each rabbit (fasted), under ketamine and xylazine (i.m.) anesthesia. The following CHECKED (X) hematology and clinical chemistry parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
-	Blood clotting measurements*		

	(Thromboplastin time) (Clotting time) (Prothrombin time)	

* Recommended for 21-day dermal toxicity studies based on Guideline 870.3200

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
-	Magnesium	X	Urea nitrogen*
X	Phosphorus	-	Total cholesterol*
X	Potassium*	-	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Bilirubin (total and direct)
-	Alkaline phosphatase (AP)*	X	Total protein*
-	Cholinesterase (ChE)	-	Triglycerides
-	Creatine phosphokinase	-	Serum protein electrophoresis
-	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
-	Glutamate dehydrogenase		
-	Sorbitol dehydrogenase*		

* Recommended for 21-day dermal toxicity studies based on Guideline 870.3200
- Not examined

6. Sacrifice and Pathology - All animals were sacrificed on schedule via an intravenous overdose of Euthanasia-5 (a barbiturate-containing solution), and were subjected to gross pathological examination. The following CHECKED (X) tissues from all animals were collected and fixed in 10% neutral buffered formalin. In addition, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
-	Tongue	-	Aorta, thoracic*	-	Brain*+
-	Salivary glands*	-	Heart*+	-	Peripheral nerve*
-	Esophagus*	-	Bone marrow*	-	Spinal cord (3 levels)*
-	Stomach*	-	Lymph nodes*	-	Pituitary*
-	Duodenum*	-	Spleen*+	-	Eyes*
-	Jejunum*	-	Thymus*+		GLANDULAR
-	Ileum*			-	Adrenal gland*+
-	Cecum*		UROGENITAL	-	Lacrimal gland
-	Colon*	XX	Kidneys*+	-	Parathyroid*
-	Rectum*	-	Urinary bladder*	-	Thyroid*
XX	Liver*+	XX[a]	Testes*+		OTHER
-	Gall bladder*	-	Epididymides*+	-	Bone (sternum and/or femur)
-	Bile duct* (rat)	-	Prostate*		Skeletal muscle
-	Pancreas*	-	Seminal vesicles*	X	Skin* (treated and untreated)
	RESPIRATORY	XX[a]	Ovaries*+	X	All gross lesions and masses*
-	Trachea*	-	Uterus*+		
-	Lung*	X	Mammary gland*		
-	Nose*				
-	Pharynx*				
-	Larynx*				

* Recommended for 21-day dermal toxicity studies based on Guideline 870.3200
\+ Organ weights required.
a Testes and ovaries were weighed but were not examined microscopically.
\- Not examined

Except for the testes and ovaries which were weighed but were not examined microscopically, the collected tissues from the control and 1035 mg/kg animals and gross lesions from all animals were processed routinely and examined microscopically.

II. RESULTS

Group 5 control animals were only used for gross and histopathology. Because the animals were older and they weighed more, the body weights, food consumption, organ weights, and clinical

pathology data were not compared to the treated groups.

A. OBSERVATIONS

1. **Clinical signs of toxicity** - No treatment-related clinical signs were observed.

2. **Mortality** - All animals survived to scheduled sacrifice.

3. **Dermal irritation** - Incidences (# affected/6 vs. 0 controls) of dermal irritation were limited to the females, and included the following (Table 2): (i) erythema at 345 (2) and 1035 mg/kg (3); (ii) edema at 345 (2) and 1035 mg/kg (1); (iii) fissuring at 345 (1) and 1035 mg/kg (2); and (iv) eschar at 1035 mg/kg (1). These findings were observed between Days 10-21. No indication of severity was included in the summary or individual data.

Table 2. Incidence (# affected/6) of dermal irritation in female rabbits dermally exposed to AC 38,555 for 6 hrs/day, 5 days/week for 3 weeks. [a]

Observation	Dose (mg/kg/day)			
	0	115	345	1035
Erythema	0	0	2	3
Edema	0	0	2	1
Fissuring	0	0	1	2
Eschar	0	0	0	1

a Data were obtained from Table 4 on page 27 of the study report.

B. BODY WEIGHT AND WEIGHT GAIN - No treatment-related effects on body weight or body weight gain were observed in either sex (Table 3).

Table 3. Mean (±SD) body weights and overall (Days 1-22) body weight gains (kg) in rabbits dermally exposed to AC 38,555 for 6 hrs/day, 5 days/week for 3 weeks. [a]

Days	Dose (mg/kg/day)							
	0	115	345	1035	0	115	345	1035
	Males				Females			
1	2.3	2.2	2.3	2.3	2.2	2.2	2.3	2.2
22	2.5	2.4	2.6	2.6	2.5	2.5	2.5	2.6
Gain (1-22)[b]	0.2	0.2	0.3	0.3	0.3	0.3	0.2	0.4

a Data were obtained from Table 1 on pages 21-22 of the study report; n=6.
b Calculated by reviewers using data within this table.

C. FOOD CONSUMPTION - No treatment-related effects on food consumption were observed in either sex. The minor increases ($p \leq 0.05$) observed in the 345 mg/kg males and females were

not dose-dependent and were not considered adverse.

D. BLOOD ANALYSES

1. Hematology - No treatment-related effects on any hematological parameter were observed. The several differences ($p \leq 0.05$) noted in various hematological parameters were not considered to be treatment-related, because they were minor, not dose-dependent, and/or were similar to pre-dosing levels.

2. Clinical chemistry - No treatment-related clinical chemistry effects were observed. The several differences ($p \leq 0.05$) noted in various clinical chemistry parameters were not considered to be treatment-related, because they were not dose-dependent or were similar to pre-dosing levels.

E. SACRIFICE AND PATHOLOGY

1. Organ weight - No treatment-related effects on absolute or relative (to body) organ weights were observed.

2. Gross pathology - Treatment-related gross lesions (# affected/6 treated vs 0 controls) were limited to foci of the treated skin in the 1035 mg/kg males (2) and the ≥345 mg/kg females (1 each dose group).

3. Microscopic pathology - At 1035 mg/kg, the following histopathological effects (# affected/6 vs 0 controls) were noted in the treated skin (Table 4): (i) minimal to mild acanthosis in the males (2) and females (4); (ii) minimal to mild subacute inflammation in the males (2) and females (1); (iii) minimal to mild edema in the males (2) and females (3); and (iv) minimal fibrosis in the males (1). Additionally, a single 345 mg/kg female displayed mild acanthosis and minimal subacute inflammation and edema.

Table 4. Selected histopathological lesions (# affected) in the treated skin of rabbits dermally exposed to AC 38,555 for 6 hrs/day, 5 days/week for 3 weeks. [a]

Parameter		Dose (mg/kg/day)							
		0	115	345	1035	0	115	345	1035
		Males				Females			
Acanthosis	Total	0	0	0	2	0	0	1	4
	Minimal	0	0	0	1	0	0	0	4
	Mild	0	0	0	1	0	0	1	0

Parameter		Dose (mg/kg/day)							
		0	115	345	1035	0	115	345	1035
		Males				Females			
Subacute Inflammation	Total	0	0	0	2	0	0	1	1
	Minimal	0	0	0	1	0	0	1	1
	Mild	0	0	0	1	0	0	0	0
Edema	Total	0	0	0	2	0	0	1	3
	Minimal	0	0	0	1	0	0	1	3
	Mild	0	0	0	1	0	0	0	0
Fibrosis, Minimal		0	0	0	1	0	0	0	0

a Data were obtained from Table 10 on pages 45-46 and from individual data on pages 140, 144, 166, and 170-174 of the study report; n=6, except for the controls n=12 (Groups 1 and 5).

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigators concluded that the dermal NOAEL was 115 mg/kg/day based on mild acanthosis, minimal subacute inflammation, and minimal edema observed in a single female at 345 mg/kg/day. The systemic NOAEL was 1035 mg/kg/day (> the limit dose).

B. REVIEWER COMMENTS - No compound-related effects on mortality, clinical signs, body weight, body weight gain, food consumption, hematology, clinical chemistry, or organ weights were observed.

In the 345 mg/kg/day group, dermal irritation characterized by erythema, edema, and fissuring was observed in 1-2 females, a gross lesion (foci) was seen in one female, and histopathological lesions in the treated skin (mild acanthosis and minimal subacute inflammation and edema) in another female.

At 1035 mg/kg/day, dermal irritation (erythema, edema, fissuring, and eschar) was also observed in 1-3 females. Gross lesions were limited to foci in the treated skin in 2 males and 1 female. Minimal to mild histopathological effects observed in the treated skin in both sexes included: (i) acanthosis (2 males and 4 females); (ii) subacute inflammation (2 males and 1 female); and (iii) edema (2 males and 3 females). Additionally, a single male displayed minimal fibrosis of the treated skin.

The systemic LOAEL was not observed and the systemic NOAEL is 1035 mg/kg/day (> the

limit dose).

The dermal LOAEL is 345 mg/kg/day based on dermal irritation, gross lesion of the treated skin, acanthosis, subacute inflammation, and edema in females. The dermal NOAEL is 115 mg/kg/day.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3200; OECD 410) for a 21-day dermal toxicity study in rabbits.

C. STUDY DEFICIENCIES - The following deficiencies were noted, but do not change the conclusions of this DER:

- Blood clotting parameters were not measured.
- The epididymides, thymus, heart, spleen, brain, adrenal gland, and uterus were not weighed.
- Numerous tissues were not examined microscopically.
- Alkaline phosphatase, sorbitol dehydrogenase, and total cholesterol were not measured.
- The rabbits were older than recommended at the initiation of dosing.
- A description of the test material was not provided.

DATA FOR ENTRY INTO ISIS

Subchronic Dermal (21 day) Study - rabbits (870.3200)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
109709	42246603	Subchronic	rabbit	21 days	dermal	dermal	115-1035	0, 115, 345, 1035	1035	Not observed	N/A	Systemic
109709	42246603	Subchronic	rabbit	21 days	dermal	dermal	115-1035	0, 115, 345, 1035	115	345	Treated skin	Dermal

DATA EVALUATION RECORD

AC 38,555 (CHLORMEQUAT CHLORIDE)

Study Type: §83-3a; Developmental Toxicity Study in Rats

Work Assignment No. 3-01-74 A; formerly 2-01-74 A (MRID 42246604)

Prepared for
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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Date: 09-21-05

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.

Signature: Steven Brecher
Date: 9/22/05

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** Karlyn
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 6/22/06
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/12/06

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700a [§83-3a]; OECD 414.

PC CODE: 018101 **DP BARCODE:** D325193
TXR#: 0054020

TEST MATERIAL (PURITY): Chlormequat chloride (AC 38,555; 66.1% a.i., doses adjusted for purity)

SYNONYMS: (2-Chloroethyl)trimethylammonium chloride; Chlormequat chloride

CITATION: Lochry, E.A. (1990) An oral developmental toxicity (embryo-fetal toxicity/teratogenicity) definitive study with AC 38,555 *in* rats. Argus Research Laboratories, Inc., Horsham, PA. Laboratory Project Id.: 101-011, December 5, 1990. MRID 42246604. Unpublished.

SPONSOR: American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 42246604), Chlormequat chloride (AC 38,555; 66.1% a.i., doses adjusted for purity; Lot # AC 6779-98A) in reverse osmosis membrane processed deionized water was administered daily via oral gavage to 25 presumed pregnant Sprague-Dawley (Crl:CD BR VAF/Plus) rats/group at a dose volume of 10 mL/kg at dose levels of 0, 30, 90, or 180 mg/kg/day from gestation day (GD) 6 through 15. All surviving dams were killed on GD 20; their fetuses were removed by cesarean section and examined.

There were no treatment-related effects on mortality or gross pathology.

At ≥ 90 mg/kg/day, clinical signs of toxicity included increased incidences of excess salivation (7-21 treated dams vs 0 controls) and chromorrhinorrhea (2-3 treated dams vs 0 controls). Clinical signs first occurred for the 90 mg/kg/day dams after the second dose (GD 7) and for the 180 mg/kg/day dams after the first dose (GD 6). Additionally at 180 mg/kg/day, decreased motor activity, tremors, ataxia, lacrimation, rales, gasping, body jerks, and increased incidences of chromodacryorrhea were observed.

At ≥ 90 mg/kg/day, maternal body weight gains were decreased ($p \leq 0.05$) during GD 6-9 (38-112%) and GD 6-12 (21-67%). Additionally at 180 mg/kg/day, body weight gains were decreased ($p \leq 0.01$) during GD 7-8 (144%), 8-9 (103%), and 9-12 (38%), resulting in decreased (36%; $p \leq 0.01$) body weight gains for the overall (GD 6-16) treatment interval. Body weights at this dose were decreased (5-9%) beginning on GD 8 and continuing until termination. During the post-treatment interval, body weight gains of the treated groups were comparable to controls. Gravid uterine weights of the treated groups were comparable to controls. Thus, body weight gains from the beginning of treatment until termination (GD 6-20) were decreased ($p \leq 0.01$) whether uncorrected (13%) or corrected (39%) for gravid uterine weight.

At 90 mg/kg/day, decreases (7-16%; $p \leq 0.05$) in absolute (g/rat/day) and relative (to body weight) food consumption (g/kg/day) were observed during GD 7-8, 6-9, and 6-12. Additionally at this dose, absolute food consumption was decreased during GD 9-12. At 180 mg/kg/day, absolute and relative food consumption were decreased (7-34%; $p \leq 0.05$) throughout the treatment interval. During the post-treatment interval, absolute food consumption was comparable to controls, and relative food consumption was increased (8%; $p \leq 0.01$) compared to controls.

The maternal LOAEL is 90 mg/kg/day based on increased incidences of excess salivation and chromorrhinorrhea and decreased body weight gains and food consumption. The maternal NOAEL is 30 mg/kg/day.

There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses. There were no treatment-related effects on the numbers of litters, live fetuses, early resorptions, late resorptions, or on the sex ratio or post-implantation loss. There were no treatment-related effects on fetal body weights or on skeletal ossification, indicating no effect on fetal growth or development. There were no treatment-related external, visceral, or skeletal malformations or variations.

The developmental LOAEL is not determined and the developmental NOAEL is 180 mg/kg/day.

This study is classified **acceptable/guideline (OPPTS 870.3700a)** and satisfies the guideline requirements for a developmental study in the rat.

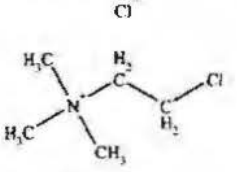
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: AC 38,555 (Chlormequat chloride)

Description:	Yellowish brown liquid
Lot/Batch #:	AC 6779-98A
Purity:	66.1% a.i. (dose formulations were adjusted for purity)
Compound Stability:	The test substance as an aqueous concentrate was stable for up to 2 years at room temperature.
CAS #of TGAI:	999-81-5
Structure:	



2. Vehicle: Reverse osmosis deionized water

3. Test animals

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD®BR VAF/Plus®)
Age/weight on GD 0:	11-12 weeks old; 227-257 g
Source:	Charles River Laboratories, Inc. (Raleigh, NC for females and Portage, MI for males)
Housing:	Individually in wire-bottomed stainless steel cages suspended above absorbent paper liners
Diet:	Certified Rodent Chow© #5002 (Ralston Purina), *ad libitum*
Water:	Reverse osmosis deionized water, with 0.6-1.0 ppm chlorine added, *ad libitum*
Environmental conditions:	
Temperature:	74 ± 6°F
Humidity:	35-70%
Air changes:	At least 10/hr
Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	Approximately 1 week

B. STUDY DESIGN

1. In life dates - Start: 07/09/90 End: 07/26/90

2. Mating - Female rats were paired (1:1) in the home cage of the male for up to four days. Each mating pair was examined daily, and positive evidence of mating was confirmed by the presence of a copulatory plug or sperm in a vaginal smear. The day on which evidence of mating was identified was designated as gestation day (GD) 0.

3. Study design - Animals were randomly assigned (stratified by body weight) to the test groups reported in Table 1.

Table 1. Study design [a]

Dose (mg/kg/day)	# Females
0	25
30	25
90	25
180	25

a Data were obtained from page 19 of the study report.

4. **Dose selection rationale** - The dose levels for the definitive study were selected based on the results of a range-finding developmental toxicity study (Protocol #971-90-107). In the range-finding study, AC 38,555 (66.1% a.i., Lot # AC 6779-98A) in reverse osmosis deionized water was administered daily via oral gavage to 8 presumed pregnant Sprague-Dawley (Crl:CD®BR VAF/Plus®) rats/group at a dose volume of 10 mL/kg at dose levels of 0, 30, 60, 120, or 240 mg/kg/day from gestation day (GD) 6 through 15. Doses were adjusted for purity. All surviving dams were killed on GD 20; their fetuses were removed by cesarean section and examined.

At ≥120 and 240 mg/kg/day, one dam/dose group died. Clinical signs of toxicity observed in the dams at these doses included decreased motor activity, excess salivation, ataxia, and tremors. Maternal body weight gains, maternal food consumption, gravid uterine weights, and fetal weights were decreased in these dose groups compared to controls. Relative (to body weight) maternal food consumption increased during the post-dosing period. Additionally at 240 mg/kg/day, an increased incidence of piloerection was noted compared to controls.

5. **Dosage preparation and analysis** - The appropriate amount of the test substance in aqueous concentrate (adjusted for purity) was measured out for each dose group, and the vehicle was added to bring the formulation up to volume to prepare dosing formulations of 3, 9, or 18 mg/mL. Dose formulations were prepared weekly (storage conditions not reported). Although triplicate samples were taken, concentration analyses were conducted on duplicate samples of each concentration taken on the first day (GD 6) and at the end (GD 13) of the dosing period. Homogeneity of the test material in the vehicle was not reported. However, the reviewers consider these data to be unnecessary because the test material was an aqueous concentrate and the vehicle used to prepare the dose formulations was water. Thus, the dose formulations were considered to be in solution instead of a suspension in which settling may be a problem. Although stability of the dose formulations was not determined, acceptable stability data were provided for the aqueous concentrate for up to 2 years at room temperature (Reg. Doc. # BASF 87/10124; Oct. 13, 1987). Again, because the vehicle was water, the stability data for the aqueous concentrate are considered acceptable.

Results -

Homogeneity: Not reported

Stability (% nominal for aqueous concentrate): 99.9% after 24 months at room temperature

Concentration (% nominal): 102-108%

The analytical data indicated that the variation between nominal and actual dosage to the animals was acceptable.

6. **Dosage administration** - Dose formulations were administered daily from GD 6-15 via oral gavage in a dose volume of 10 mL/kg. Doses were adjusted daily based on the individual body weight recorded immediately prior to intubation.

C. OBSERVATIONS

1. **Maternal observations and evaluations:** All dams were checked daily for clinical observations and abortions, premature deliveries, and deaths were recorded daily from GD 0-20. Animals were also observed for signs of toxicity approximately one hour following dosing. It was stated that body weights (g), absolute (g/rat/day) and relative to body weight (g/kg/day), and food consumption data were recorded on GD 0 and daily from GD 6-20. However, data were not presented for every day during the treatment and post-treatment periods. The net body weight (body weight on GD 20 minus the gravid uterine weight) and net body weight gains for GD 0-20, 6-20, and 16-20 (body weight gain for each interval minus gravid uterine weight) were calculated for each gravid female at the time of the cesarean section. Rats that were found dead were necropsied that same day to attempt to determine the cause of death and to examine the uterine contents. On GD 20, all surviving dams were euthanized by carbon dioxide asphyxiation and were subjected to a gross necropsy. The uterus and ovaries were excised, and the uterine weight was recorded. The number of corpora lutea in each ovary were counted. The number and location of all implantations, live and dead fetuses, and early and late resorptions in the uterus were recorded. The uterus of any rat that appeared non-pregnant was stained with 10% ammonium sulfide solution to confirm pregnancy status according to the Salewski technique.

2. **Fetal evaluations:** Each fetus was weighed, sexed, and examined for external abnormalities. Live fetuses were euthanized by fixation in the appropriate fixative. Approximately one-half of the fetuses in each litter were examined for visceral abnormalities using a modified Wilson's sectioning technique. The remaining fetuses were cleared, stained with Alizarin Red S, and examined for skeletal abnormalities.

D. DATA ANALYSIS

1. **Statistical analyses:** The following statistical tests were applied to the data:

Parameter	Statistical test
Maternal body weight Maternal body weight gain Maternal food consumption Gravid uterine weight Litter averages for fetal body weights	Bartlett's test was conducted to determine if variances were homogeneous. If variances were homogeneous ($p>0.05$), analysis of variance (ANOVA) was performed, followed by pair-wise comparison of treated groups with controls using Dunnett's test, if ANOVA was significant ($p\leq0.05$). If variances were not homogeneous ($p\leq0.05$), Kruskal-Wallis test was used, when less than or equal to 75% ties were present, followed by pair-wise comparison of treated groups with controls using Dunn's test, if
Cesarean section parameters	Kruskal-Wallis test was used, when less than or equal to 75% ties were present, followed by pair-wise comparison of treated groups with controls using Dunn's test, if Kruskal-Wallis was significant ($p\leq0.05$). When more than 75% ties were present, Fisher's Exact test was used.
Maternal and fetal incidence	Variance test for homogeneity of the binomial distribution

Significance was denoted at $p\leq0.05$ and $p\leq0.01$. Before proceeding with parametric analyses, the assumptions of normal distribution of the data should have been verified. Otherwise, the statistical methods were considered appropriate.

2. **Indices:** No indices were reported. Pre- and post-implantation losses were calculated by the reviewers from the individual data according to the following formulae:

Pre-implantation loss (%) = (#corpora lutea - #implantations)/#corpora lutea x 100
Post-implantation loss (%) = (#implantations - #live fetuses)/#implantations x 100

3. **Historical control data:** Historical control data, comprising 90-135 studies conducted from 1987-1989, were provided for cesarean section parameters (90 studies), maternal necropsy (135 studies), and for external, visceral, and skeletal alterations in the fetuses (75 studies).

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical observations:** Approximately one hour after dose administration on GD 6, one 180 mg/kg/day dam (#7725) was found dead. Necropsy of this animal revealed a perforated esophagus and red fluid in the thoracic cavity, indicating gavage error. This animal was pregnant, and its litter of 15 embryos appeared normal for that stage of development. All other animals survived to scheduled termination.

At ≥ 90 mg/kg/day, clinical signs of toxicity included increased incidences of excess salivation (7-21 treated dams vs 0 controls) and chromorrhinorrhea (2-3 treated dams vs 0 controls; Table 2). Clinical signs first occurred for the 90 mg/kg/day dams after the second dose (GD 7) and for the 180 mg/kg/day dams after the first dose (GD 6). Additionally at 180 mg/kg/day, the following clinical signs of toxicity were observed (# treated dams vs 0 controls, unless otherwise noted): (i) decreased motor activity (19); (ii) tremors (16); (iii) ataxia (7); (iv) lacrimation (6); (v) rales (2); (vi) gasping (1); (vii) chromodacryorrhea (3 treated vs 1 control); and (viii) body jerks (1). Significance ($p \leq 0.01$) was attained for incidences of excess salivation, decreased motor activity, tremors, ataxia, and lacrimation at 180 mg/kg/day. There were no other treatment-related clinical signs. Two 30 mg/kg/day dams (#7669 and 7670) exhibited excess salivation, red substance on nose/mouth, and/or gasping for 1 or 2 days; it was stated that these signs were related to injury during dose administration.

Table 2. Clinical signs of toxicity [# animals (total # animal days)] observed in the maternal rats at one hour post-dosing [a]

Interval	Dose (mg/kg/day)			
	0	30	90	180
Excess salivation	0 (0)	2 (3)[b]	7 (10)	21 (59)**
Chromorrhinorrhea	0 (0)	0 (0)	2 (2)	3 (3)
Decreased motor activity	0 (0)	0 (0)	0 (0)	19 (45)**
Tremors	0 (0)	0 (0)	0 (0)	16 (31)**
Ataxia	0 (0)	0 (0)	0 (0)	7 (8)**
Lacrimation	0 (0)	0 (0)	0 (0)	6 (9)**
Rales	0 (0)	0 (0)	0 (0)	2 (5)
Gasping	0 (0)	1 (2)[b]	0 (0)	1 (1)
Chromodacryorrhea	1 (1)	0 (0)	1 (1)	3 (3)
Body jerks	0 (0)	0 (0)	0 (0)	1 (1)

a Data were obtained from Table 1 on page 40 of the study report; n = 25.
b It was stated that excess salivation and gasping in this animal were associated with injuries due to dose administration.

2. <u>Body weight</u>: Selected maternal body weight gain data are presented in Table 3. At ≥90 mg/kg/day, maternal body weight gains were decreased ($p \leq 0.05$) during GD 6-9 (38-112%) and GD 6-12 (21-67%). Additionally at 180 mg/kg/day, body weight gains were decreased ($p \leq 0.01$) during GD 7-8 (144%), 8-9 (103%), and 9-12 (38%), resulting in decreased (36%; $p \leq 0.01$) body weight gains for the overall (GD 6-16) treatment interval. Furthermore, body weights at this dose were decreased (5-9%) beginning on GD 8 and continuing until termination. During the post-treatment interval, body weight gains of the treated groups were comparable to controls. Gravid uterine weights of the treated groups were comparable to controls. Thus, body weight gains from the beginning of treatment until termination (GD 6-20) were decreased ($p \leq 0.01$) whether uncorrected (13%) or corrected (39%) for gravid uterine weight. There were no other

treatment-related effects on body weights or body weight gains. Although body weight gains were decreased ($p \leq 0.05$) in the 30 mg/kg/day dams during GD 6-9, this decrease was considered unrelated to treatment because it was incidental and not dose-related.

Table 3. Selected mean (± SD) maternal body weights and body weight gains [a]

Interval	Dose in mg/kg/day			
	0	30	90	180 [b]
GD 0	241.4 ± 8.2	240.6 ± 8.6	240.2 ± 8.4	240.1 ± 8.3
GD 6	278.8 ± 11.2	279.5 ± 12.0	276.4 ± 14.3	274.1± 12.1
GD 8	286.4 ± 13.1	285.3 ± 14.5	280.9±14.7	273.2 ± 13.9** (↓5%)
GD 12	313.0 ± 16.3	310.0 ± 15.6	303.4 ± 16.3	285.9 ± 16.2** (↓9%)
GD 18	371.1 ± 26.0	371.8 ± 20.2	367.8 ± 22.0	349.3 ± 20.9** (↓6%)
GD 20	407.5 ± 28.7	411.8 ± 22.7	404.9 ± 25.0	386.2 ± 24.8** (↓5%)
Gravid uterine weight	83.1 ± 19.7	88.2 ± 12.6	89.8 ± 14.7	83.8 ± 13.0
Corrected body weight	324.4 ± 16.1	323.5 ± 18.3	315.1 ± 15.2	302.4 ± 19.8** (↓7%)
Pre-treatment: GD 0-6	37.3 ± 8.1	38.9 ± 7.0	36.1 ± 9.5	34.0 ± 6.8
Treatment: GD 7-8	4.3 ± 4.1	2.8 ± 4.6	2.2 ± 5.2	-1.9 ± 4.7** (↓144)
GD 8-9	5.9 ± 4.6	2.0 ± 7.1	4.0 ± 4.2	-0.2 ± 6.2** (↓103)
GD 6-9	13.5 ± 5.5	7.9 ± 9.8* (↓41)	8.4 ± 8.1* (↓38)	-1.6 ± 9.1** (↓112)
GD 6-12	34.2 ± 7.9	30.5 ± 7.4	27.0 ± 9.6* (↓21)	11.3 ± 10.7** (↓67)
GD 9-12	20.7 ± 6.3	22.6 ± 5.4	18.5 ± 6.0	12.9 ± 6.5** (↓38)
Overall (GD 6-16)	62.2 ± 12.4	60.5 ± 12.9	58.8 ± 11.2	39.9 ± 15.0** (↓36)
Post-treatment: GD 16-20	66.5 ± 12.1	71.7 ± 10.2	69.8 ± 10.0	71.7 ± 10.8
Uncorrected gain GD 6-20	128.8 ± 21.9	132.3 ± 14.7	128.5 ± 17.2	111.6 ± 18.1** (↓13)
Gravid uterine weight	83.1 ± 19.7	88.2 ± 12.6	89.8 ± 14.7	83.8 ± 13.0
Corrected gain GD 6-20	45.6 ± 8.5	44.0 ± 12.3	38.7 ± 9.8	27.8 ± 13.8** (↓39)

a Data were obtained from Tables 3 and 4 on pages 42-44 of the study report; n = 22-25. Percent difference from controls is included in parentheses.

* Significantly different from the control group at $p \leq 0.05$

** Significantly different from the control group at $p \leq 0.01$

3. **Food consumption:** Selected maternal food consumption data are presented in Table 4. At 90 mg/kg/day, decreases (7-16%; $p \leq 0.05$) in absolute (g/rat/day) and relative (to body weight) food consumption (g/kg/day) were observed during GD 7-8, 6-9, and 6-12. Additionally at this

34

dose, absolute food consumption was decreased during GD 9-12. At 180 mg/kg/day, absolute and relative food consumption was decreased (7-34%; p≤0.05) throughout the treatment interval. During the post-treatment interval, absolute food consumption was comparable to controls, and relative food consumption was increased (8%; p≤0.01) compared to controls.

Table 4. Selected mean (± SD) maternal food consumption [a]

Interval	Dose in mg/kg/day			
	0	30	90	180 [b]
Absolute (g/animal/day)				
Pre-treatment: GD 0-6	21.1 ± 2.1	21.9 ± 2.2	21.0 ± 2.7	20.6 ± 1.7
Treatment: GD 7-8	22.2 ± 2.7	21.4 ± 3.7	18.7 ± 3.0** (↓16)	15.4 ± 3.1** (↓31)
GD 8-9	22.0 ± 2.9	20.5 ± 5.3	20.0 ± 3.2	14.6 ± 4.2** (↓34)
GD 6-9	21.8 ± 2.1	21.0 ± 3.0	19.6 ± 2.5** (↓10)	15.9 ± 2.9** (↓27)
GD 6-12	22.8 ± 2.2	22.5 ± 2.6	20.7 ± 2.5* (↓9)	16.6 ± 3.0** (↓27)
GD 9-12	23.8 ± 2.6	23.9 ± 2.5	21.9 ± 2.8* (↓8)	17.3 ± 3.5** (↓27)
GD 12-16	24.7 ± 2.6	25.6 ± 2.9	24.7 ± 2.4	21.0 ± 3.6** (↓15)
Overall (GD 6-16)	23.5 ± 2.2	23.7 ± 2.6	22.3 ± 2.3	18.4 ± 3.1** (↓22)
Post-treatment: GD 16-20	27.0 ± 2.4	27.7 ± 2.3	26.9 ± 2.5	27.6 ± 3.1
Relative to body weight (g/kg/day)				
Pre-treatment: GD 0-6	81.1 ± 6.8	84.2 ± 6.6	81.0 ± 8.1	80.0 ± 4.9
Treatment: GD 7-8	77.9 ± 8.7	75.2 ± 11.4	66.4 ± 8.6** (↓15)	56.1 ± 10.2** (↓28)
GD 8-9	75.9 ± 9.3	71.3 ± 16.9	70.5 ± 9.2	53.1 ± 14.0** (↓30)
GD 6-9	76.4 ± 6.0	73.8 ± 8.7	69.7 ± 6.8** (↓9)	57.7 ± 9.0** (↓24)
GD 6-12	77.4 ± 5.4	76.8 ± 6.5	72.0 ± 6.1* (↓7)	59.6 ± 8.8** (↓23)
GD 12-16	75.6 ± 5.3	78.7 ± 7.2	77.6 ± 4.8	70.3 ± 9.5* (↓7)
Overall (GD 6-16)	76.7 ± 4.8	77.6 ± 6.3	74.3 ± 4.6	64.0 ± 8.4** (↓17)
Post-treatment: GD 16-20	72.5 ± 3.6	74.2 ± 4.2	73.1 ± 5.0	78.8 ± 6.8** (↑8)

a Data were obtained from Tables 5 and 6 on pages 45-46 of the study report; n = 22-24. Percent difference from controls is included in parentheses.

* Significantly different from the control group at p≤0.05

** Significantly different from the control group at p≤0.01

4. **Gross pathology:** There were no treatment-related macroscopic findings.

5. **Cesarean section data:** Cesarean section data are presented in Table 5. There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses. There were no treatment-related effects on the numbers of litters, live fetuses, early resorptions, late resorptions, or on the fetal body weights, sex ratio, or post-implantation loss.

37

Table 5. Cesarean section observations [a]

Observation	Dose (mg/kg/day)			
	0	30	90	180
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	24	22	24	25
Pregnancy Rate (%)	96	88	96	100
# Nonpregnant [b]	1	3	1	0
Maternal Wastage				
# Died	0	0	0	1
# Died Pregnant	0	0	0	1
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea [c]	445	407	458	431
Corpora Lutea/Dam	18.5 ± 3.8	18.5 ± 2.1	19.1 ± 2.3	18.0 ± 2.7
Total # Implantations [c]	357	353	395	364
(Implantations/Dam)	14.9 ± 3.5	16.0 ± 2.4	16.4 ± 2.3	14.9 ± 2.3
Total # Litters	24	22	24	24
Total # Live Fetuses	335	334	368	337
(Live Fetuses/Dam)	14.0 ± 3.4	15.2 ± 2.4	15.3 ± 2.5	14.0 ± 2.2
Total # Dead Fetuses	0	0	0	0
(Dead Fetuses/Dam)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total # Resorptions [b]	22	19	27	21
Early	22	19	26	21
Late	0	0	1	0
Total Resorptions/Dam [b]	0.9	0.9	1.1	0.9
Early	0.9 ± 0.8	0.9 ± 1.0	1.1 ± 1.0	0.9 ± 1.1
Late	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0
Complete Litter Resorption	0	0	0	0
Mean (±SD) Fetal Weight (g) Males	3.86 ± 0.49	3.77 ± 0.27	3.75 ± 0.24	3.77 ± 0.22
Females	3.56 ± 0.24	3.54 ± 0.31	3.55 ± 0.22	3.56 ± 0.20
Sex Ratio (% Male)	50.8 ± 17.4	50.4 ± 12.8	47.1 ± 12.2	55.2 ± 15.3
Pre-implantation Loss (%) [b, d]	19.8	13.3	13.8	15.5
Post-implantation Loss (%) [b, e]	6.2	5.4	6.8	7.4

a Data were obtained from Table 1 on page 40 and Tables 7 and 8 on pages 47 and 48 of the study report.
b Calculated by the reviewers from data presented in this table.
c Tabulated by the reviewers from individual data presented in Table 18 on pages 80-83 of the study report.
d Pre-implantation loss (%) = (#corpora lutea - #implantations)/#corpora lutea x 100
e Post-implantation loss (%) = (#implantations - #live fetuses)/#implantations x 100

B. DEVELOPMENTAL TOXICITY

1. **External examination:** No external malformations or variations were noted.

2. Visceral examination: There were no treatment-related visceral malformations or variations. The only visceral finding was slight dilatation of the renal pelvis in a single 90 mg/kg/day fetus (Table 6).

Table 6. Visceral findings [# fetuses (litters) affected] [a]

Observations	Dose (mg/kg/day)			
	0	30	90	180
# Fetuses (# litters) examined	160 (23)	162 (22)	178 (24)	162 (24)
Kidney(s) - slight dilatation of the renal pelvis	0 (0)	0 (0)	1 (1)	0 (0)

a Data were obtained from Table 11 on page 51 of the study report.

3. Skeletal examination: Skeletal findings are listed in Table 7. One fetus at 180 mg/kg/day (#7708-13) had a duplicated manubrium, xiphoid, and sternebrae. Although these malformations were not noted in the concurrent or historical controls, this finding is considered incidental, as it only occurred in a single fetus. Incidences of all other skeletal alterations were unrelated to dose.

Table 7. Skeletal findings [% fetuses (% litters) affected] [a]

Observations		Dose (mg/kg/day)				Historical Controls [b]
		0	30	90	180	
# Fetuses (# litters) examined		175 (24)	172 (22)	190 (24)	175 (24)	7003 (820)
Manubrium -	duplicated	0 (0)	0 (0)	0 (0)	0.6 (4.2)[c]	Not observed
Xiphoid -	duplicated	0 (0)	0 (0)	0 (0)	0.6 (4.2)[c]	Not observed
Sternebrae -	duplicated	0 (0)	0 (0)	0 (0)	0.6 (4.2)[c]	Not observed
	delayed ossification - total	4.0 (20.8)	2.9 (9.1)	1.6 (8.3)	0.6 (4.2)	0-6.5 (0-44.4)
	incompletely ossified	2.3 (8.3)	1.2 (4.5)	0.5 (4.2)	0.6 (4.2)	—
	not ossified	1.7 (12.5)	2.3 (9.1)	1.0 (8.3)	0 (0)	—
Vertebrae - thoracic centrum, bifid		0 (0)	1.2 (9.1)	0 (0)	0.6 (4.2)	0-2.6 (0-17.4)[d]
Ribs - cervical rib at the 7th cervical vertebra		0.6 (4.2)	0 (0)	0 (0)	0 (0)	0-2.4 (0-30)
Pelvis - pubes,	*incompletely ossified*	2.8 (16.7)	1.7 (9.1)	1.6 (8.3)	0 (0)	0-5.2 (0-25.0)
ischia,	incompletely ossified	0.6 (4.2)	0 (0)	0.5 (4.2)	0 (0)	0-1.4 (0-12.5)

a Data were obtained from Table 12 on pages 52-54 of the study report.
b Historical control data were obtained from pages 309-310 of the study report.
c Findings denoted by this suprascript (i.e., duplicated manubrium, xiphoid, and sternebrae) were noted in a single fetus (#7708-13).
d Reported more generally as "cervical ribs present" in the historical control data.
— Not provided

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: It was concluded that the maternal LOAEL was 90 mg/kg/day based on increased incidences of excess salivation and chromorrhinorrhea and on decreased body weight gains and food consumption. Additionally at 180 mg/kg/day, further clinical signs of toxicity (i.e, tremors, ataxia, lacrimation, rales, gasping, chromodacryorrhea, body jerks, and decreased motor activity) were observed, and body weights were decreased. The developmental LOAEL was not observed.

B. REVIEWER COMMENTS

1. Maternal toxicity: There were no effects of treatment on mortality or gross pathology. At ≥90 mg/kg/day, clinical signs of toxicity included increased incidences of excess salivation (7-21 treated dams vs 0 controls) and chromorrhinorrhea (2-3 treated dams vs 0 controls). Clinical signs first occurred for the 90 mg/kg/day dams after the second dose (GD 7) and for the 180 mg/kg/day dams after the first dose (GD 6). Additionally at 180 mg/kg/day, decreased motor activity, tremors, ataxia, lacrimation, rales, gasping, body jerks, and increased incidences of chromodacryorrhea were observed.

At ≥90 mg/kg/day, maternal body weight gains were decreased ($p \leq 0.05$) during GD 6-9 (↓38-112%) and GD 6-12 (↓21-67%). Additionally at 180 mg/kg/day, body weight gains were decreased ($p \leq 0.01$) during GD 7-8 (↓144%), 8-9 (↓103%), and 9-12 (↓38%), resulting in decreased (↓36%; $p \leq 0.01$) body weight gains for the overall (GD 6-16) treatment interval. Furthermore, body weights at this dose were decreased (↓5-9%) beginning on GD 8 and continuing until termination. During the post-treatment interval, body weight gains of the treated groups were comparable to controls. Gravid uterine weights of the treated groups were comparable to controls. Thus, body weight gains from the beginning of treatment until termination (GD 6-20) were decreased ($p \leq 0.01$) whether uncorrected (↓13%) or corrected (↓39%) for gravid uterine weight.

At 90 mg/kg/day, decreases (↓7-16%; $p \leq 0.05$) in absolute (g/rat/day) and relative (to body weight) food consumption (g/kg/day) were observed during GD 7-8, 6-9, and 6-12. Additionally at this dose, absolute food consumption was decreased during GD 9-12. At 180 mg/kg/day, absolute and relative food consumption was decreased (↓7-34%; $p \leq 0.05$) throughout the treatment interval. During the post-treatment interval, absolute food consumption was comparable to controls, and relative food consumption was increased (↑8%; $p \leq 0.01$) compared to controls.

The maternal LOAEL is 90 mg/kg/day based on increased incidences of excess salivation and chromorrhinorrhea and decreased body weight gains and food consumption. The maternal NOAEL is 30 mg/kg/day.

2. Developmental toxicity

a. Deaths/Resorptions: There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses. There were no treatment-related effects on the numbers of litters, live fetuses, early resorptions, late resorptions, or on the sex ratio or post-implantation loss.

b. Altered Growth: There were no treatment-related effects on fetal body weights or on skeletal ossification, indicating no effect on fetal growth or development.

c. Developmental Variations: There were no treatment-related external, visceral, or skeletal variations.

d. Malformations: There were no treatment-related external, visceral, or skeletal malformations.

The developmental LOAEL is not determined and the developmental NOAEL is 180 mg/kg/day.

This study is classified **acceptable/guideline (OPPTS 870.3700a)** and satisfies the guideline requirements for a developmental study in the rat.

C. STUDY DEFICIENCIES: No study deficiencies were noted.

DATA FOR ENTRY INTO ISIS

Developmental Study - rats (870.3700a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
109709	42246604	developmental	rat	GD 6-15	oral	gavage	30-180	0, 30, 90, 180	30	90	clinical signs, decr. BWG, FC	Maternal
109709	42246604	developmental	rat	GD 6-15	oral	gavage	30-180	0, 30, 90, 180	180	Not observed		Developmental

42

DATA EVALUATION RECORD

CHLORMEQUAT CHLORIDE

Study Type: §83-3b; Developmental Toxicity Study in Rabbits

Work Assignment No. 3-01-104 A (MRID 46715205)

Prepared for
Health Effects Division
Office of Pesticide Programs
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** KJ
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 6/27/06
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 7/12/06
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OPPTS 870.3700b [§83-3b]; OECD 414.

PC CODE: 018101 **DP BARCODE:** D325193
TXR#: 0054020

TEST MATERIAL (PURITY): Chlormequat chloride technical (99% a.i.)

SYNONYMS: 2-chloro-*N,N,N*-trimethylethanaminium chloride; 2-chloroethyltrimethyl ammonium chloride; chlorocholine chloride

CITATION: Hofmann, H.T., and Werkle, J. (1979) Study of the prenatal toxicity of 2-chloroethyltrimethylammonium chloride (chlormequat chloride) on rabbits. BASF AG, Ludwigshafen/Rhein, Germany. Laboratory Project ID.: BASF Registration Document No. 1979/051, March 16, 1979. MRID 46715205. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46715205), Chlormequat chloride (99% a.i.; Lot # 78/557/78/639) in distilled water was administered daily via oral gavage (10 mL/kg) to groups of 14-21 artificially inseminated Himalayan (ChBB:HM) rabbits/dose at dose levels of 0, 1.5, 3.0, 6.0, or 12.0 mg/kg/day on gestation days (GD) 6-18. All surviving does were killed on GD 28; their fetuses were removed by cesarean section and examined.

Maternal toxicity

There were no treatment-related effects observed on mortality, clinical signs, or gross pathology.

At 12.0 mg/kg/day, body weight gains were decreased ($p \leq 0.01$) during GD 6-12 (330%), resulting in an overall (GD 0-28) decrease (not significant [NS]) in body weight gains (32%). However, a recovery in mean body weight gain was observed during GD 12-18, and

individual body weight gain data revealed erratic body weight gains at 12.0 mg/kg/day; a common finding in this species. In addition, an outlier was identified while examining the individual body weight data. There were no treatment-related effects on body weight gains observed at 1.5, 3.0, and 6.0 mg/kg/day. Maternal absolute body weights for all treatment groups were comparable to the control group throughout the study. Mean food consumption was decreased ($p \leq 0.05$) in all treatment groups during GD 6-18 (12-17%), resulting in decreased (not significant; NS) overall (GD1-28) food consumption (8%). The decrease in food consumption observed in treated groups can not be verified since individual food consumption data was not reported; therefore, it can not be determined whether the decrease in food consumption is indeed an effect of treatment.

The maternal LOAEL is not determined and the maternal NOAEL is 12.0 mg/kg/day.

Developmental toxicity

There were no treatment-related external, visceral, or skeletal malformations or variations observed. In addition, there were no effects of treatment observed on numbers of litters, live fetuses, resorptions (early, late, or complete litter), sex ratio, or post-implantation loss. No treatment-related effects were observed on fetal body weight or crown-rump length, or placental weight.

The developmental LOAEL is not determined and the developmental NOAEL is 12.0 mg/kg/day.

This developmental toxicity study in the rabbit is classified **acceptable/non-guideline** and does not satisfy the guideline requirement (OPPTS 870.3700b; OECD 414) for a developmental toxicity study in rabbits. The frequency of preparation of the test formulations and homogeneity, stability, and concentration analyses were not provided. In addition, a LOAEL was not determined and the animals could have tolerated a higher dose.

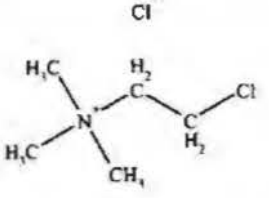
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Flagging statements were provided. A Quality Assurance statement was not provided. It was stated that this study did not meet the requirements of 40 CFR Part 160; however, this study was conducted prior to the adoption of GLP standards (40 CFR Part 160; November 29, 1983).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Chlormequat chloride technical

Description:	Not provided
Lot/batch #:	78/557/78/639
Purity:	99% a.i.
Compound stability:	Not provided
CAS #of TGAI:	999-81-5
Structure:	



2. **Vehicle:** distilled water

3. **Test animals:**

Species:	Rabbit	
Strain:	Himalayan (ChBB:HM)	
Age/weight at study initiation:	25-39 weeks old; 1.912-2.682 kg	
Source:	Thomae (Biberach, Germany)	
Housing:	Individually in wire cages	
Diet:	Ssniff-K standardized pelleted diet (Plange, Kraftfutterwerk Soest GmbH), 130 g daily	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±2EC
	Humidity:	55±5%
	Air changes:	Not provided
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Eight days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: 09/06/78 End: 12/14/78

2. **Mating:** Rabbits were artificially inseminated at the performing laboratory. One hour prior to insemination, the does were given an intravenous injection of 40 I.U. of Primogonyl. The day of insemination was designated as gestation day (GD) 0.

3. **Animal Assignment:** Animals were randomly assigned to the dose groups as indicated in Table 1.

TABLE 1: Animal Assignment					
Dose (mg/kg/day)	0	1.5	3.0	6.0	12.0
Number of Females	15	15	21	21	14

4. **Dose selection rationale:** A dose-selection rationale was not provided.

5. **Dosage preparation and analysis:** The frequency of preparation of the test formulations was not provided. It was stated that since the test substance was hygroscopic, a 0.7631% stock solution was prepared after the second treatment, and this stock solution was diluted for the various dose preparations. The test compound was dissolved in distilled water. Homogeneity, stability, and concentration analyses were not provided; however, chlormequat chloride is known to be water soluble. Therefore, it is reasonable to assume the test formulations were homogeneous.

   Since analytical data were not provided, it was impossible to determine if the variation between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** Test formulations were administered from GD 6-18 via oral gavage in a dose volume of 10 mL/kg. Individual dose volumes were based on the body weight recorded on GD 0 and were administered at approximately the same time each day. It was stated that untreated animals served as controls; however, it was not clear if these animals were completely untreated or were gavaged with distilled water (vehicle controls).

C. OBSERVATIONS

1. **Maternal observations and evaluations:** All dams were checked daily for morbidity, mortality, clinical signs of toxicity, and abortion. Body weights were recorded prior to treatment (not reported) and on GD 0, 6, 12, 18, and 28. Food consumption was recorded daily throughout the study and reported (total g and g/animal/day) for GD 1-6, 6-12, 12-18, 18-28, and for the overall (GD 1-28) study. Animals that aborted were killed on the day of abortion by an intravenous injection of Nembutal. All surviving rabbits were killed on GD 28 by a blow to the base of the skull followed by exsanguination.

   All dams, including those found dead or that aborted, were subjected to necropsy. The uterus was excised, and gravid uterine weights were recorded. The number of corpora lutea was recorded. The number of all live and dead fetuses, the number and location of implantation sites, and the number of early[1], intermediate[2], and late[3] resorptions were also recorded. The

[1] Defined by the Sponsor as "recognizable as brown spots on the mucosa of the uterus" after ammonium sulfide staining and "recognizable to the naked eye as yellowish brown spots."
[2] Defined by the Sponsor as "dead embryos and embryos undergoing resorption in which no parts of the body were recognizable macroscopically.
[3] Defined by the Sponsor as "dead embryos and embryos undergoing resorption in which individual parts of the body were recognizable macroscopically.

uteri of apparently nonpregnant does were examined for implantation sites by staining with a 10% ammonium sulfide solution (method of Salewski).

2. **Fetal evaluations:** Each fetus was examined for external abnormalities. The weight, crown-rump length, and placental weight of each viable fetus were recorded. All fetuses were necropsied and then X-rayed in two planes (laterally and anteriorly/posteriorly) for assessment of skeletal abnormalities. After being X-rayed, the heads of all the fetuses were removed and fixed in Bouin's solution. Following fixation, transverse sections of the heads were prepared and examined (method of Wilson).

D. DATA ANALYSIS:

1. **Statistical analyses:** The following statistical tests were applied to the data:

Parameter	Statistical test
Body weight, body weight gain, food consumption, and fetal body weights, crown-rump length, and placental weights	A trend analysis in the sense of the generalization of the t-test indicated by Williams
Conception rate, viable fetuses per pregnant animal, dead implantations per pregnant animal, dead animals, litters with anomalous fetuses per litters overall, litters which have fetuses with variations and retardations per litters overall	Fisher's exact test
Implants and percent viable and dead embryos per pregnant animal, and anomalies, variations, and retardations as a percent of viable fetuses per litter	Mann-Whitney U test with Walter's binding correction, and if necessary, the continuity correction according to Yates

Significance was denoted at p#0.05 and p#0.01. The statistical methods were considered appropriate.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

Conception rate = # of animals pregnant/# animals inseminated x 100

The following indices were calculated by the reviewers:

Sex ratio (% male fetuses) = # live males/# live fetuses x 100

Pre-implantation loss (%) = (# corpora lutea - # implantations)/# corpora lutea x 100

Post-implantation loss (%) = (# implantations - # live fetuses)/# implantations x 100

3. **Historical control data:** Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical observations:** There were no treatment-related mortalities. One control doe, one 3.0 mg/kg/day doe, and two 6.0 mg/kg/day does died. The cause of death for the 3.0 and one of the 6.0 mg/kg/day animals was given as "severe agitation during gavage." The cause of death for the control doe and the other 6.0 mg/kg/day doe was not provided.

   Two 6.0 mg/kg/day does and one 12.0 mg/kg/day doe aborted. One 6.0 mg/kg/day doe (# 71) aborted on GD 20; the other 6.0 mg/kg/day doe (# 68) aborted on GD 25. The 12.0 mg/kg/day doe (#82) aborted on GD 27 after being observed with severe diarrhea. These abortions were considered incidental.

   There were no treatment-related clinical signs of toxicity. Individual animals in the control, 1.5, 3.0, and 6.0 mg/kg/day groups were observed to have temporary diarrhea. One 6.0 mg/kg/day doe and one 12.0 mg/kg/day doe presented with salivation, accelerated respiration, and apathy for one day each on GD 13 and 14, respectively. No other clinical signs of toxicity were observed.

2. **Body weight:** Body weight gains were decreased ($p \leq 0.01$) in the 12.0 mg/kg/day does during GD 6-12 (↓330%), resulting in decreased (not significant [NS]) overall (GD 0-28) body weight gains (↓32%; Table 2). No treatment-related effects were observed on body weights or body weight gains in any other group.

TABLE 2 Mean (±SD) maternal body weight gain (g) [a]

Interval	Dose (mg/kg/day)				
	0	1.5	3.0	6.0	12.0
Pre-treatment GD 0-6	-7.67±30.68	-1.00±50.92	-15.20±67.60	-5.87±57.03	16.67±39.29
Treatment GD 6-12	20.33±40.85	-0.57±45.79	-7.20±77.84	-16.67±56.18	-46.67±62.52** (↓330)
GD 12-18	89.50±56.07	54.86±61.82	32.86±78.75	39.73±65.48	78.67±108.60
Post-treatment GD 18-28	120.67±65.43	106.86±92.11	198.43±61.91	117.54±95.55	102.67±147.89
Overall (GD 0-28)	222.83±97.53	160.14±122.60	209.86±121.11	160.15±151.06	151.33±248.43 (↓32)

a Data were obtained from Table 5 on page 46 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n=12-15

** Significantly different from controls; $p \leq 0.01$

3. **Food consumption:** Food consumption data are presented in Table 3. At 12.0 mg/kg/day, food consumption was decreased ($p \leq 0.05$) during GD 6-18 (↓12-17%), resulting in decreased (NS) overall (GD 1-28) food consumption (↓8%).

Food consumption was also decreased ($p \leq 0.05$) at 1.5, 3.0, and 6.0 mg/kg/day during GD 6-12 (↓12-17%), and at 3.0 and 6.0 mg/kg/day during GD 12-18 (↓25-35%), resulting in decreased (NS) overall food consumption in these dose groups (↓10-13%). The decrease in food consumption observed in treated groups can not be verified since individual food consumption data was not reported.

TABLE 3 Mean (±SD) cumulative maternal food consumption (g) [a]

Interval	Dose (mg/kg/day)				
	0	1.5	3.0	6.0	12.0
Pre-treatment GD 1-6	448.02±103.10	434.48±88.93	405.49±142.27	336.20±132.85	475.99±140.76
Treatment GD 6-12	603.18±78.40	502.94±109.75* (↓17)	532.82±95.52* (↓12)	513.44±78.01* (↓15)	532.54±85.33* (↓12)
GD 12-18	569.06±93.98	492.86±124.08	372.04±170.05* (↓35)	428.83±184.52* (↓25)	471.70±123.83* (↓17)
Post-treatment GD 18-28	990.56±140.70	909.84±198.49	991.84±106.42	919.05±140.15	920.38±314.12
Overall (GD 1-28)	2361.23±265.00	2128.16±312.78 (↓10)	2113.65±278.18 (↓10)	2053.89±294.39 (↓13)	2183.25±431.39 (↓8)

a Data were obtained from Table 1 on page 42 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses. n=12-15

* Significantly different from controls; $p \leq 0.05$

4. **Gross pathology:** No treatment-related effects were observed during necropsy.

5. **Cesarean section data:** Cesarean section data are presented in Table 4. No effects of treatment were observed on numbers of litters, live fetuses, dead fetuses, resorptions (early, late, or complete litter), fetal body weight or crown-rump length, placental weight, sex ratio, or post-implantation loss.

   At 12.0 mg/kg/day, increases ($p \leq 0.05$) were observed in mean fetal weight for females (↑17%) and for the combined sexes (↑9%), in mean fetal length for females (↑6%) and for the combined sexes (↑4%), and in mean placental weight for females (↑21%). However, increases in these parameters were not considered adverse.

TABLE 4 Cesarean section observations [a]

Observation	Dose (mg/kg/day)				
	0	1.5	3.0	6.0	12.0
No. Animals assigned (mated)	15	15	21	21	14
No. Animals pregnant	12	14	15	15	12
Pregnancy rate (%)	80.0	93.3	71.4	71.4	85.7
No. Nonpregnant [c]	3	1	6	6	2
Maternal wastage					
No. died	0	0	1	2	0
No. Died pregnant	0	0	1	0	0
No. Died nonpregnant	0	0	0	2	0
No. Aborted	0	0	0	2	1
No. Premature delivery	0	0	0	0	0
Total No. corpora lutea	119	107	121	107	81
Corpora lutea/Dam	9.92	7.64	8.64	8.23	7.36
Total No. implantations	63	57	77	56	55
(Implantations/Dam)	5.25	4.07	5.50	4.31	5.00
Total No. litters [b]	12	14	13	13	11
Total No. live fetuses	59	49	68	50	47
(Live fetuses/Dam)	4.92	3.50	4.86	3.85	4.27
Total No. dead fetuses	0	1	1	0	0
(Dead fetuses/Dam) [c]	0.00	0.07	0.07	0.00	0.00
Total No. resorptions	4	7	8	6	8
Early [d]	4	7	6	6	8
Late	0	0	2	0	0
Resorptions/Dam [c]	0.33	0.50	0.53	0.40	0.67
Early [c, d]	0.33	0.50	0.40	0.40	0.67
Late [c]	0	0	0.13	0	0
Complete litter resorption [b]	0	0	1	0	0
Mean fetal weight (g)	37.08±3.33	37.23±3.08	35.59±3.52	35.68±3.97	40.35±2.19* (↑9)
Males	38.37±3.58	36.58±4.93	35.83±3.82	36.57±4.29	40.49±2.40
Females	34.40±4.01	36.84±2.48	34.65±4.00	33.71±6.16	40.20±2.59** (↑17)
Mean fetal length (cm)	7.97±0.47	7.89±0.37	7.89±0.36	8.04±0.44	8.32±0.32* (↑4)
Males	8.03±0.38	7.90±0.48	7.90±0.32	8.25±0.48	8.40±0.44
Females	7.79±0.52	7.75±0.42	7.83±0.51	7.60±0.71	8.29±0.39* (↑6)
Mean placental weight (g)	5.73±0.79	6.05±1.03	5.43±0.71	5.97±1.26	6.30±0.53
Males	5.99±0.69	5.86±1.07	5.46±0.69	6.07±1.25	6.37±0.72
Females	5.20±0.81	5.76±0.86	5.30±1.13	5.41±1.05	6.27±0.52* (↑21)
Sex ratio (% male) [e]	47.5	46.9	51.5	46.0	53.2
Pre-implantation loss (%) [c, f]	47.1	46.7	36.4	47.7	32.1
Post-implantation loss (%) [c, g]	6.3	14.0	11.7	10.7	14.5

a Data were obtained from Tables 16-22 and 23-25 on pages 57-63 and 64-66 of the study report.
b Tabulated by reviewers
c Calculated by reviewers from data presented in this table
d Early (recognizable as brown spots on the mucosa of the uterus after ammonium sulfide staining; recognizable to the naked eye as yellowish brown spots) and intermediate (dead embryos and embryos undergoing resorption in which no parts of the body were recognizable macroscopically) resorptions were combined by the reviewers.
e Calculated by reviewers
f Pre-implantation loss (%) = (# corpora lutea - # implantations)/# corpora lutea x 100
g Post-implantation loss (%) = (# implantations - # live fetuses)/# implantations x 100
* Significantly different from controls; p≤0.05
** Significantly different from controls; p≤0.01

B. DEVELOPMENTAL TOXICITY

1. **External examination:** No treatment-related external findings were observed (Table 5a). A single 1.5 mg/kg/day fetus was observed with both cleft palate and pseudoankylosis (2.0% fetuses; 7.1% litters), both malformations, compared to 0 concurrent controls. These findings were considered incidental. There were no other external findings.

TABLE 5a. External malformations [% fetuses (litters) affected] [a]

Observations	Dose (mg/kg/day)				
	0	1.5	3.0	6.0	12.0
No. Fetuses(litters) examined	59 (12)	49 (14)	68 (13)	50 (13)	47 (11)
Cleft palate [b]	0 (0)	2.0 (7.1)	0 (0)	0 (0)	0 (0)
Pseudoankylosis [b]	0 (0)	2.0 (7.1)	0 (0)	0 (0)	0 (0)

a Percent incidence calculated by the reviewers from data obtained from page 31, Table 37 on page 78, and Table 53 on page 94 of the study report.
b Observed in the same fetus

2. **Visceral examination:** No treatment-related visceral findings were observed (Table 5b). One 1.5 mg/kg/day fetus was observed to have a cleft palate (2.0% fetuses; 7.1% litters), a malformation; and one 3.0 mg/kg/day fetus was noted to have retinal folds (1.5% fetuses; 7.7% litters), a variation, both compared to 0 concurrent controls. These findings were considered incidental. There were no other visceral findings.

TABLE 5b. Visceral examinations [% fetuses (litters) affected] [a]

Observations [b]	Dose (mg/kg/day)				
	0	1.5	3.0	6.0	12.0
No. Fetuses(litters) examined	59 (12)	49 (14)	68 (13)	50 (13)	47 (11)
Malformations					
Cleft palate	0 (0)	2.0 (7.1)	0 (0)	0 (0)	0 (0)
Variations					
Retinal folds	0 (0)	0 (0)	1.5 (7.7)	0 (0)	0 (0)

a Percent incidence calculated by the reviewers from data obtained from Tables 37, 38, 53, and 54 on pages 78, 79, 94, and 95 of the study report.

3. **Skeletal examination:** No treatment-related skeletal findings were observed (Table 5c). Absent sternebrae, a variation, was noted in the 1.5 (34.7% fetuses; 78.6% litters), 3.0 (36.8% fetuses; 69.2% litters), 6.0 (38.0% fetuses; 69.2% litters), and 12.0 (48.9% fetuses; 72.7% litters) mg/kg/day groups compared to concurrent controls (39.0% fetuses; 75.0% litters). Although fetal incidence was increased at 12.0 mg/kg/day, the litter incidence was approximately the same as concurrent controls; therefore, this finding was considered incidental. Asymmetrical sternebrae, a variation, was observed in the 1.5 (2.0% fetuses; 7.1% litters), 6.0 (4.0% fetuses; 15.4% litters), and 12.0 (2.1% fetuses; 9.1% litters) mg/kg/day groups compared to concurrent controls (1.7% fetuses; 8.3% litters). Partially ossified sternebrae, a retardation, was noted in the 1.5 (53.1% fetuses; 85.7% litters), 3.0 (38.2% fetuses; 76.9% litters), 6.0 (42.0% fetuses; 69.2% litters), and 12.0 (31.9% fetuses;

90.0% litters) compared to concurrent controls (37.3% fetuses; 58.3% litters). These finding were not dose-dependent, and were not considered treatment-related. Bilateral accessory ribs, a variation, was observed in one 6.0 mg/kg/day fetus, and unilateral accessory ribs, a variation, was noted in one 3.0 mg/kg/day fetus, both compared to 0 concurrent controls. Both of these findings were considered incidental. There were no other skeletal findings.

TABLE 5c. Skeletal examinations [% fetuses (litters) affected] [a]

Observations [b]	Dose (mg/kg/day)				
	0	1.5	3.0	6.0	12.0
No. Fetuses(litters) examined	59 (12)	49 (14)	68 (13)	50 (13)	47 (11)
Variations					
Absent sternebrae	39.0 (75.0)	34.7 (78.6)	36.8 (69.2)	38.0 (69.2)	48.9 (72.7)
Asymmetrical sternebrae	1.7 (8.3)	2.0 (7.1)	0 (0)	4.0 (15.4)	2.1 (9.1)
Accessory ribs, bilateral	0 (0)	0 (0)	0 (0)	2.0 (7.7)	0 (0)
Accessory ribs, unilateral	0 (0)	0 (0)	1.5 (7.7)	0 (0)	0 (0)
Retardations					
Partially ossified sternebrae	37.3 (58.3)	53.1 (85.7)	38.2 (76.9)	42.0 (69.2)	31.9 (90.9)

a Percent incidence calculated by the reviewers from data obtained from Tables 38, 40, 42, 44, 46, 49-50, 52, and 54 on pages 79, 81, 83, 85, 87, 90-91, 93, and 95 of the study report.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS CONCLUSIONS: There were no treatment-related effects observed on mortality, clinical signs, or gross pathology. There were temporary decreases in body weight gain at 12 mg/kg/day. The food consumption of the animals in all test groups was temporarily affected. There were no treatment-related effects on developmental parameters. The LOAEL for does and fetuses is expected to be above 12.0 mg/kg/day.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** The reviewer agrees with the conclusions of the study investigator. There were no treatment-related effects observed on mortality, clinical signs, or gross pathology.

   At 12.0 mg/kg/day, body weight gains were decreased ($p \leq 0.01$) during GD 6-12 (330%), resulting in an overall (GD 0-28) decrease (not significant [NS]) in body weight gains (32%). However, a recovery in mean body weight gain was observed during GD 12-18, and individual body weight gain data revealed erratic body weight gains at 12.0 mg/kg/day; a common finding in this species. In addition, an outlier was identified while examining the individual body weight data. There were no treatment-related effects on body weight gains observed at 1.5, 3.0, and 6.0 mg/kg/day. Maternal absolute body weights for all treatment groups were comparable to the control group throughout the study. Mean food consumption was decreased ($p \leq 0.05$) in all treatment groups during GD 6-18 (12-17%), resulting in decreased (not significant; NS) overall (GD1-28) food consumption (8%). The decrease in food consumption observed in treated groups can not be verified since individual food

consumption data was not reported; therefore, it can not be determined whether the decrease in food consumption is indeed an effect of treatment.

The maternal LOAEL is not determined and the maternal NOAEL is 12.0 mg/kg/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** No effects of treatment were observed on numbers of litters, live fetuses, dead fetuses, resorptions (early, late, or complete litter), sex ratio, or post-implantation loss.

   b. **Altered growth:** No effects of treatment were observed on fetal body weight or crown-rump length, or placental weight. There were no treatment-related skeletal retardations.

   c. **Developmental variations:** No treatment-related external, visceral, or skeletal variations were observed.

   d. **Malformations:** No treatment-related external, visceral, or skeletal malformations were observed.

The developmental LOAEL is not determined and the developmental NOAEL is 12.0 mg/kg/day.

C. **STUDY DEFICIENCIES:** The following deficiencies were noted:

- The frequency of preparation of the test formulations and homogeneity, stability, and concentration analyses were not provided. Since analytical data were not provided, it was impossible to determine if the animals received the targeted dose.
- Does were dosed from GD 6-18, instead of from implantation to one day prior to the expected day of parturition. However, this study was performed prior to the implementation of the US EPA Health Effect Guidelines (OPPTS 870.3700b, August 1998).
- A dose-selection rationale was not provided.
- Doses of the test formulations were based on the individual body weights recorded on GD 0, and were not adjusted during the study.
- Historical control data were not provided.

DATA EVALUATION RECORD

CHLORMEQUAT CHLORIDE

Study Type: §83-4; Multigeneration Reproduction Study in Rats

Work Assignment No. 3-1-104 B (MRID 46715206)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** Karlyn
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 6/27/06
EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/14/06
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study - [rat] OPPTS 870.3800 [§83-4]; OECD 416.

PC CODE: 018101 **DP BARCODES:** D325193
TXR#: 0054020

TEST MATERIAL (PURITY): Chlormequat chloride (67.4% a.i., dose levels adjusted for purity)

SYNONYMS: 2-chloro-*N,N,N*-trimethylethanaminium

CITATION: Hellwig, J., and B. Hildebrand (1993) Reproduction toxicity study with Chlormequat-chloride in rats: continuous dietary administration over 2 generations (2 litters in the first and 1 litter in the second generation). Department of Toxicology, BASF Aktiengesellschaft, Ludwigshafen/Rhein, FRG. Laboratory Report No(s).: 71R0580/87099, August 30, 1993. MRID 46715206. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a multigeneration reproduction toxicity study (MRID 46715206), chlormequat chloride (67.4% a.i., dose levels adjusted for purity; Batch No. 82-0767) was administered continuously in the diet to Wistar rats (24 rats/sex/dose) at dose levels of 0, 300, 900, or 2700 ppm (approximately equivalent to 0, 28.9, 86.4, and 254.6 mg/kg/day in males and 0, 30.8, 93.4, and 279.3 in females). The P animals were given test article diet formulations for 10 weeks prior to mating and throughout mating to produce the F1a litters, and throughout gestation and lactation for females; they were subsequently mated again to produce a second litter (F1b retained only until weaning). After weaning, F1a animals (24/sex/dose) were selected to become the parents of the F2 generation and were given the same concentration test formulation as their parents. F1 animals were given test formulations for 14 weeks prior to mating and throughout mating to produce the F2 litters, and throughout gestation and lactation for females. In addition to the typical parameters examined in a reproductive toxicity study, clinical chemistry parameters were examined in 12 female F1 rats/dose

No treatment-related adverse effect was observed in the parents on mortality, food consumption, mating or gestation indices, gestation duration, clinical chemistry, or on gross or histological pathology.

At 900 ppm in the P generation dams, mean body weight gain was decreased during lactation of the first litter by 52% ($p \leq 0.01$) and second litter by 72% (NS; Table 3a). These decreases in body weight gains were not associated with decreased body weights at 900 ppm.

At 2700 ppm, increased incidences of tremor and hypersensitivity were observed during lactation. Decreased body weights and body weight gains were observed in the females of the P and F1 generations, generally throughout the study but usually most severe during lactation. Decreased body weight gains were observed during pre-mating in the P generation only, and in gestation and lactation in both generations.

The LOAEL for parental toxicity is 2700 ppm (approximately equivalent to 254.6 mg/kg/day in males and 279.3 mg/kg/day in females), based on decreased body weights and body weight gains of the P and F1 generations, and increased incidences of tremor and hypersensitivity. The NOAEL is 900 ppm (approximately equivalent to 86.4mg/kg/day in males and 93.4 mg/kg/day in females).

No treatment-related effect was observed on male or female mating indices.

Fertility was decreased in the 2700 ppm group at each mating. The fertility index was 75-83% in the treated groups compared to concurrent controls (96-100%) and historical controls (88-100%). However, fertility was reevaluated in those animals that were not observed to be fertile, and most of the animals were proven fertile. In the P generation, only one 2700 ppm female did not prove its fertility. In the F1 generation, one 300 ppm male was not reevaluated due to a technical error; one 900 ppm female, one 2700 ppm male, and one 2700 ppm female did not prove to be fertile. However, an effect of treatment may have been diluted by mating treated rats with controls for this re-evaluation.

The LOAEL for reproductive performance is 2700 ppm (approximately equivalent to 254.6 mg/kg/day in males and 279.3 mg/kg/day in females), based on decreased fertility indices. The NOAEL is 900 ppm (approximately equivalent to 86.4 mg/kg/day in males and 93.4 mg/kg/day in females).

No treatment-related effect was observed on number of pups born dead; live birth, viability, or lactation indices; sex ratio; acoustic startle and pupil constriction responses; or gross or histological pathology.

At 2700 ppm, decreased ($p \leq 0.01$) mean litter size was observed at Days 0 (34%) and 4 before culling (30%) in the F1a litter. On Days 0 and 4 (before culling), mean litter size was also decreased (NS) in the F1b (9-14%) and F2 (16-17%) litters.

At 2700 ppm, decreased ($p \leq 0.01$) body weights were observed in the F1a generation on PND 14

and 21 (13-18%). Decreased ($p \leq 0.05$) body weights were observed in the F2 generation on PND 7, 14, and 21 (12-26%).

At 2700 ppm in the F1a, F1b, and F2 generations, fewer ($p \leq 0.01$) pups were noted with pinna unfolding (80-84% treated vs 91-98% controls), auditory canal opening (84-91% vs 97-99%), and eye opening (77-81% vs 94-100%). In addition, fewer ($p \leq 0.05$) pups demonstrated the gripping reflex at 2700 ppm in the F1a generation (95% treated vs 100% controls), but the frequency was similar to controls in the F1b and F2 generations.

The LOAEL for offspring toxicity is 2700 ppm (approximately equivalent to 254.6 mg/kg/day in males and 279.3 mg/kg/day in females), based on decreased mean litter size, body weight, as well as delayed development. The NOAEL is 900 ppm (approximately equivalent to 86.4 mg/kg/day in males and 93.4 mg/kg/day females).

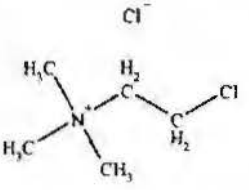
This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

COMPLIANCE: Signed and dated Data Confidentiality, GLP compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Chlormequat chloride

Description:	Liquid
Batch No.:	82-0767
Purity:	67.4% a.i.; dose levels were adjusted for purity
Compound Stability:	
CAS # of TGAI:	999-81-5
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat	
Strain:	Wistar (*Chbb = THOM (SPF)*)	
Age at treatment initiation:	(P) 35±1 days. (F1) ≤14 days	
Group mean weight at treatment initiation:	(P) 146-147 g males; 126-128 g females. (F1) 74-102 g in males; 69-94 g in females	
Source:	Karl THOMAE (Biberach an der Riss, Germany)	
Housing:	Individually in type DK III stainless steel wire mesh cages. Makrolon type M III cages during mating and from GD 18 until PND 14. Cellulose wadding was provided as bedding toward the end of pregnancy.	
Diet:	Ground Kliba maintenance diet rat/mouse/hamster GLP 343 meal (KLINGEN-TALMUHLE AG, Kaiseraugst, Switzerland), *ad libitum* until 16 hours prior to sacrifice (except the infertile rats were not fasted)	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature**	20-24EC
	Humidity	30-70%
	Air changes	Not reported
	Light cycle	12 hours light/12 hours dark
Acclimation period:	9 Days	

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: Generally, each P and F1 female was paired overnight with one male from the same group (chosen by lot) until mating was confirmed or for a maximum of 3 weeks. Vaginal examinations were performed daily during the cohabitation period. Positive evidence of mating was determined by the presence of sperm in the vaginal smear. The day on which positive evidence of mating was found was designated as gestation day (GD) 0. Mating of the same partners was avoided during the second mating of the P animals, as was F1 sibling mating. If the mating resulted in no offspring, the animals were mated with fertile (proven) controls (1:1) for up to 3 weeks to verify infertility.

2. **Study schedule:** At least 70 days after the beginning of treatment, P rats were mated to produce the F1a litters. The P rats were mated again at least 10 days after the last weaning of the F1a generation to produce the F1b litters (retained until weaning). Prior to weaning of the F1a offspring, one pup/sex/litter (as near as possible) was randomly selected to continue on study as parents for the F2 generation. At least 98 days after formation of the F1a generation parental animals, these rats were mated to produce the F2 litters. Males were sacrificed after their final scheduled mating. The P generation dams were sacrificed after weaning of the F1b generation (PND 22), and the F1 generation dams were sacrificed after weaning of the F2 generation (PND 22). At the time of mating, the P animals were approximately 105 days old at the first mating and 136 days old at the second mating. The F1 animals were 98 days old when mated.

3. **Animal assignment:** The P animals were randomly assigned, stratified by body weight, to the test groups shown in Table 1.

TABLE 1. Animal assignment

Test group	Dose in diet [a] (ppm)	Animals/group			
		P Males	P Females	F1 Males	F1 Females
Control	0	24	24	24	24
Low (LDT)	300	24	24	24	24
Mid (MDT)	900	24	24	24	24
High (HDT)	2700	24	24	24	24

[a] Diets were administered from beginning of the study until 16 hours prior to sacrifice

4. **Dose-selection rationale:** The doses for the current study were based on the results of an earlier reproduction study, 4-week range-finding study, 18-month chronic toxicity study, and 24-month carcinogenicity study. In the reproduction study, no effects were observed on the parents or the F1 and F2 generations at doses up to 900 ppm. In the 18- and 24-month chronic studies, decreased body weights, body weight gains, and food consumption were observed in both sexes at 2811 ppm. Decreased body weight gain in both sexes was also observed in the range-finding study at 3000 ppm; and decreased body weight gain, food consumption, and creatinine in both sexes, deteriorated general state of health in both sexes, and decreased total protein in males and urea in females were noted at 4500 ppm.

5. **Dosage preparation and analysis:** The test diets were prepared at intervals of not more than 32 days. The appropriate amount of test material was mixed with a small amount of diet to form a premix. The premix was further diluted with diet to achieve the appropriate doses. Prior to animal treatment, the test compound stability in a 500 ppm dietary formulation was tested for up to 32 days at room temperature. Six samples each from 500 and 4500 ppm dietary formulations were analyzed to verify homogeneity prior to animal treatment. The concentration of each dietary formulation was analyzed in duplicate once prior to treatment and at approximately 3 month intervals thereafter.

Results:

Homogeneity (% coefficient of variation): 1.8-2.8%

Stability (% of nominal): 98.4%

Concentration (range of % nominal): 90.9-105.3%

The data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. **Parental animals:** All animals were observed daily for mortality, moribundity, and clinical signs of toxicity. It was not stated that detailed physical examinations were conducted. The littering behavior of the dams was also inspected on weekdays (except holidays) in the afternoons. Generally, body weights for parents were measured at randomization, at the beginning of treatment, weekly throughout the study, and at termination. Body weights of dams were weighed on GD 0, 7, 14, and 20. Females with litters were weighed on LD 0, 4, 7, 14, and 21. For both sexes, generally, food consumption (g/animal/day) was reported weekly throughout premating and for the overall pre-mating period in the P (Weeks 0-10) and F1 (Weeks 0-14) generations. Food consumption was measured in dams during GD 0-7, 7-14, and 14-20, and LD 0-4, 4-7, and 7-14. The group mean compound intake (mg/kg bw/day) values were calculated from the food consumption and body weight data, and was reported weekly throughout pre-mating. Estrous cyclicity and sperm parameters were not examined. Blood was collected from the retroorbital plexus of 12 female F1 parental animals/dose at PND 141 and PND 152, and the following CHECKED (X) parameters, including serum and erythrocyte cholinesterase, were examined.

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Creatinine
X	Magnesium	X	Urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose (not fasted)
	ENZYMES (more than 2 hepatic enzymes)	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total protein
X	Cholinesterase (ChE) (serum and RBC)	X	Triglycerides
-	Creatine phosphokinase	-	Serum protein electrophores
-	Lactic acid dehydrogenase (LDH)	-	Albumin/globulin
X	Alanine aminotransferase (ALT/ SGPT)		
X	Aspartate aminotransferase (AST/ SGOT)		
-	Gamma glutamyltransferase (GGT)		
-	Sorbitol dehydrogenase		
-	Glutamate dehydrogenase		

X Examined
- Not examined

2. **Litter observations:** According to the report, the following litter observations (X) were made (see Table 2).

TABLE 2. F1/ F2 Litter Observations [a]

Observation	Time of observation (lactation day)					
	Day 0	Day 4 [b]	Day 4 [c]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
External alterations	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Sex of each pup (M/F)	X	-	-	-	-	X
Developmental stages [d]	-	X	-	-	X	-
Behavioral tests [e]	-	-	-	-	X	X

[a] Information obtained from pages 49-52 in the study report.
[b] Before standardization (culling)
[c] After standardization (culling)
[d] Pinna unfolding was checked at PND 4, opening of the auditory canal at PND 13, and opening of eyes at PND 15
[e] Gripping reflex was measured at PND 13±1, acoustic startle reflex at PND 21±1, and pupillary reflex at PND 20±1
X Observed
- Not observed

Additionally, pups were examined daily for mortality and clinical signs of toxicity. On PND 4, litters of 9 or more pups were standardized to a maximum of 8 pups/litter (4 pups/sex/litter, as nearly as possible).

3. **Postmortem observations**

1) **Parental animals:** All surviving adults, along with those that died or were killed *in extremis*, were decapitated under CO_2 anesthesia, and were subjected to gross necropsy. Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera. Infertile animals were sacrificed after fertility was reevaluated and then were examined grossly and histologically. The uteri of females were examined for live and dead implantations. Early implantations were determined according to the method of Salewski.

The following tissues (X) were prepared for microscopic examination. No organs were weighed:

X	Ovaries	X	Testes
X	Uterus	X	Epididymides
X	Vagina/cervix	X	Prostate
X	Pituitary gland	X	Seminal vesicles
X	Lesions	X	Coagulation glands

The collected organs were fixed in 4% formaldehyde solution. The tissues from the controls and the 2700 ppm groups were processed, stained with hematoxylin and eosin, and microscopically examined. All gross lesions and all tissue samples from animals that died intercurrently or were sacrificed *in extremis* were also examined.

2) **Offspring:** All pups (except F1 parents) were sacrificed by means of CO_2 at culling (PND 4) or after weaning (PND 21). Pups were examined grossly for external and internal abnormalities. All pups without any notable findings or abnormalities were discarded after their macroscopic evaluation. If abnormalities were found in the daily clinical observations or notable gross findings, additional examination was carried out when deemed appropriate. This examination included skeletal staining according to modified Dawson's method and further processing of the head according to Wilson's method.

D. **DATA ANALYSIS**

1. **Statistical analyses:** Food consumption, body weight, body weight gain, and clinical chemistry data were analyzed by one-way ANOVA followed by Dunnett's test. Developmental stages, gripping and pupillary reflex, acoustic startle reflex, number of live and dead pups, male and female mating index, male and female fertility index, gestation index, live birth index, viability index, and lactation index were analyzed using Fisher's exact test. Data were analyzed for significant differences at the 5% and 1% levels of probability.

2. ***Indices***

Reproductive indices: The following reproductive/viability indices were calculated by the performing laboratory from breeding and parturition records of animals in the study:

Male mating index (%) = # of males with confirmed mating/# placed with females x 100
Female mating index (%) = # of females with confirmed mating/# placed with males x 100

Male fertility index (%) = # siring litters/# placed with females x 100
Female fertility index (%) = # pregnant/# mated x 100

Gestation index (%) = # females with live litters born/# pregnant females x 100

Live birth index (%) = # of liveborn pups at birth/total number of pups born x 100

Offspring viability indices: The following viability indices were calculated by the performing laboratory from lactation records of litters in the study:

Viability (%) = # pups alive on PND 4 (pre-cull)/# pups born alive x 100

Lactation (%) = # pups alive at PND 21/# pups alive on PND 4 (post-cull) x 100

3. **Historical control data:** The following historical control data for the Wistar rat were provided: (i) maternal body weight during gestation and lactation (n=778-780); (ii) reproduction and litter data; (iii) pup weights; (iv) pup physical and reflex data; and (v) pup necropsy data. Data for the pups were from 9 studies, comprising 21-25 litters per generation (151-379 pups/litter; 754 litters with 9647 pups).

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical signs:** There were no mortalities in the P and F1 parental animals related to the test compound. One 300 ppm P female died on Day 163 and one 900 ppm F1 female died on Day 127 due to difficulties in giving birth. Additionally in the F1 controls, a male was sacrificed moribund on Day 139, and a female died on Day 147. Treatment-related clinical signs observed in the P generation included tremor in the 2700 ppm females during Weeks 26-27 (5-6 rats/week) and LD 4-21 (3-20 rats/day), and hypersensitivity during lactation of the F1b litter on Days 4-21 (3-4 rats/day). Tremor was also observed in the F1 dams during lactation on Days 3-24 (4-18 rats/day). No other clinical sign was considered related to treatment.

2. **Body weight, body weight gain, food consumption, and food efficiency:** At 900 ppm in the P generation dams, mean body weight gain was decreased during lactation of the first litter by 52% ($p \leq 0.01$) and second litter by 72% (NS; Table 3a). These decreases in body weight gains were not associated with decreased body weights at 900 ppm.

   At 2700 ppm, decreased ($p \leq 0.05$) body weights and body weight gains were observed in the females of the P generation, generally throughout the study. Decreased ($p \leq 0.05$) body weights were observed during pre-mating (↓5-6%), during gestation of the F1a litter (↓5-11%), during lactation of the F1a litter (↓12-16%), after weaning of the F1a litter (↓8-10%), during gestation of the F1b litter (↓8-10%), and during the lactation of the F1b litter (↓10-17%). Decreased body weight gains ($p \leq 0.05$) were noted during pre-mating (↓11%), during gestation of the F1a litter (↓23%), during lactation of the F1a litter (↓143%), during gestation of the F1b litter (↓10%), and during lactation of the F1b litter (↓223%). An initial, transient decrease in body weight (Weeks 1-2, ↓6%; $p \leq 0.01$) and body weight gain (Week 0-1; ↓21%; $p \leq 0.01$) was observed in P generation males.

   An initial, transient decrease (↓7-15%; $p \leq 0.01$) in food consumption was noted during the first 2 weeks in the P males and during the first week in the P females. No decrease ($p \leq 0.05$) in overall food consumption was noted in the P generation animals. A decrease (not statistically significant [NS]) was observed in the 2700 ppm females during lactation of the F1a litter (↓22%) and F1b litter (↓19%).

TABLE 3a. Mean (±SD) body weight and food consumption in the P generation [a]

Observations/study week		Dose group (ppm)			
		0	300	900	2700
Males					
Body weight (g)	Week 0	146.8±7.6	145.7±7.4	146.4±7.4	145.6±7.1
	Week 26	528.1±39.6	544.3±53.2	552.9±60.4	523.0±48.2
Weight gain (g)	Weeks 0-26	381.3±35.9	398.6±50.1	406.5±60.7	377.4±45.1
Grand mean food consumption (g/animal/day)	Weeks 0-10	26.0±0.7	26.9±0.8	27.0±0.9	25.4±1.7
Females during pre-mating					
Body weight (g)	Week 0	126.5±5.3	127.6±5.1	126.8±5.2	126.5±5.2
	Week 2	174.8±10.4	177.8±9.0	178.6±7.7	166.4±13.2* (↓5)
	Week 7	240.9±19.2	243.5±14.2	247.8±16.6	227.4±20.7* (↓6)
	Week 10	261.1±23.3	263.4±17.6	270.3±18.6	246.7±22.3
Weight gain (g)	Weeks 0-10	134.6±20.9	135.8±15.0	143.4±16.7	120.1±20.4* (↓11)
Grand mean food consumption (g/animal/day)	Weeks 0-10	19.6±0.4	20.2±0.3*	20.7±0.4**	19.5±0.9
Females during gestation of the F1a litter					
Body weight (g)	Day 0	259.8±22.9	261.3±17.2	268.3±16.2	247.3±20.2
	Day 14	319.7±31.2	320.3±20.2	332.0±16.5	302.6±21.4* (↓5)
	Day 20	390.8±44.1	386.2±30.8	398.5±26.0	348.3±23.5** (↓11)
Weight gain (g)	Days 0-20	131.0±27.0	124.9±18.9	130.2±18.7	101.1±22.1** (↓23)
Grand mean food consumption (g/animal/day)	Days 0-20	24.5±1.6	24.5±1.2	25.5±1.4	24.4±1.7
Females during lactation of the F1a litter					
Body weight (g)	Day 0	301.9±31.7	303.1±21.5	315.6±20.1	286.1±26.9
	Day 4	314.2±27.4	313.1±21.3	323.8±19.5	275.9±29.3** (↓12)
	Day 14	338.0±26.4	336.0±21.5	342.5±19.6	282.9±26.9** (↓16)
	Day 21	323.5±26.6	323.4±20.2	325.9±18.9	276.8±26.1** (↓14)
Weight gain (g)	Days 0-21	21.6±13.4	20.3±12.2	10.3±12.4** (↓52)	-9.3±8.5** (↓143)
Grand mean food consumption (g/animal/day)	Days 0-14	43.8±12.6	45.5±12.3	46.6±13.7	34.0±10.6 (↓22)
Females after weaning of the F1a litter					
Body weight (g)	Week 18	303.7±25.0	304.9±18.9	312.4±17.6	274.0±28.5** (↓10)
	Week 19	307.2±27.6	309.9±20.8	315.7±21.7	281.6±27.8** (↓8)
Weight gain (g)	Weeks 18-19	3.5±6.1	5.0±7.3	3.3±7.1	7.6±4.8
Grand mean food consumption (g/animal/day)	Weeks 18-19	NR	NR	NR	NR
Females during gestation of the F1b litter					
Body weight (g)	Day 0	309.0±27.9	310.3±20.5	318.9±19.8	279.3±19.6** (↓10)
	Day 14	363.4±36.7	364.9±24.1	375.2±25.7	335.1±24.3** (↓8)
	Day 20	446.9±50.3	443.1±27.4	454.3±34.1	403.0±29.3** (↓10)
Weight gain (g)	Days 0-20	137.9±27.8	132.9±13.0	135.4±22.2	123.7±14.2 (↓10)
Grand mean food consumption (g/animal/day)	Days 0-20	26.1±1.2	25.7±0.7	26.6±0.8	25.6±0.8
Females during lactation of the F1b litter					
Body weight (g)	Day 0	347.0±34.2	344.4±24.9	355.9±25.1	312.9±25.1** (↓10)
	Day 21	358.6±30.3	355.3±20.1	359.1±23.1	298.5±20.9** (↓17)
Weight gain (g)	Days 0-21	11.6±15.2	10.9±12.2	3.2±13.6 (↓72)	-14.3±16.1** (↓223)
Grand mean food consumption (g/animal/day)	Days 0-14	45.8±12.7	46.1±12.1	45.9±12.6	37.1±12.2 (↓19)

[a] Data obtained from pages 114-137 in the study report.

* Statistically different from control, $p<0.05$.

** Statistically different from control, $p<0.01$.

NR Not reported

In the F1 parental rats, decreased (↓8-25%; p≤0.01) body weights were observed throughout the study (Table 3b). Body weight gains were decreased (p≤ 0.05) during the first 4 weeks in the 2700 ppm males resulting in an overall (Weeks 0-21) decrease of 7%. Decreased (p≤0.05) body weights and body weight gains were observed in the 2700 ppm females, generally throughout the study. Decreased (p≤0.05) body weights were observed throughout pre-mating (↓9-24%), gestation (↓8-11%), and lactation (↓10-19%). Decreased (p≤0.05) body weight gains were observed for the gestation (↓18%) and lactation (↓238%) periods.

A decrease (p≤0.05) in overall food consumption for each period was not observed in the F1generation animals, except for a minor decrease in males (↓8% for Weeks 0-14). Decreases (NS) were observed in the 2700 ppm females throughout lactation (↓25-28%; p≤ 0.01), resulting in a 27% decrease for the overall lactation period NS, n=3). Although food efficiency was not reported, the effect on body weight gain without a clear effect on food consumption suggests that food efficiency was decreased.

TABLE 3b. Mean (±SD) body weight and food consumption in the F1 parental rats [a]

Observations/study week		Dose group (ppm)			
		0	300	900	2700
Males					
Body weight (g)	Week 0	99.2±15.5	102.5±9.7	99.1±9.8	74.5±17.4** (↓25)
	Week 21	499.8±58.2	517.4±40.4	539.5±45.0*	447.5±54.9** (↓10)
Weight gain (g)	Weeks 0-21	400.7±50.4	415.0±37.1	440.3±44.1**	373.0±42.3 (↓7)
Grand mean food consumption (g/animal/day)	Weeks 0-14	26.3±1.8	27.1±1.9	27.7±2.2	24.1±3.0* (↓8)
Females during pre-mating					
Body weight (g)	Week 0	91.5±11.6	93.9±8.6	89.6±9.2	69.3±15.9** (↓24)
	Week 14	271.5±26.2	282.6±19.0	285.7±22.1	246.3±21.3** (↓9)
Weight gain (g)	Weeks 0-14	180.1±24.9	188.7±19.6	196.1±21.5*	177.1±12.0
Grand mean food consumption (g/animal/day)	Weeks 0-14	19.9±0.6	21.1±0.8*	21.0±1.1*	19.3±1.6
Females during gestation					
Body weight (g)	Day 0	270.2±25.5	278.2±20.4	278.2±20.1	247.7±19.3** (↓8)
	Day 20	383.4±42.3	389.5±30.5	393.4±26.8	340.7±31.0** (↓11)
Weight gain (g)	Day 0-20	113.2±25.00	111.3±25.5	115.2±20.8	93.0±21.7* (↓18)
Grand mean food consumption (g/animal/day)	Days 0-20	23.9±1.2	25.0±1.2	25.2±1.1	22.8±0.9
Females during lactation					
Body weight (g)	Day 0	311.3±26.6	314.8±23.0	318.2±21.2	279.4±21.9** (↓10)
	Day 14	335.6±27.3	338.8±21.1	334.9±21.1	271.0±21.0** (↓19)
	Day 21	322.9±27.9	333.4±22.9	334.9±20.2	263.3±20.8** (↓18)
Weight gain (g)	Days 0-21	11.6±18.6	18.6±15.0	16.7±16.2	-16.0±12.4** (↓238)
Food consumption (g/animal/day)	Days 4-7	41.7±5.2	41.7±8.9	41.4±8.4	31.3±3.8** (↓25)
Food consumption (g/animal/day)	Days 7-14	56.8±8.8	52.5±12.3	52.4±11.8	40.7±5.2** (↓28)
Grand mean food consumption (g/animal/day)	Days 0-14	42.4±14.0	41.1±11.7	41.0±11.6	31.0±9.9 (↓27)

[a] Data obtained from pages 184-201 in the study report.
* Statistically different from control, p<0.05.
** Statistically different from control, p<0.01.

3. **Test substance intake:** Based on food consumption, body weight, and nominal dietary concentration, actual doses are presented in Table 4. The actual doses are expressed as mean daily mg test substance/kg body weight during the 10-week pre-mating period for the P generation and 14-week pre-mating period for the F1generation The values for the P generation are considered to be representative of the test substance intake for the entire study.

TABLE 4. Mean test substance intake during premating (mg/kg body weight/day) [a]

	Male			Female		
	300 ppm	900 ppm	2700 ppm	300 ppm	900 ppm	2700 ppm
P	28.9±10.5	86.4±30.7	254.6±73.4	30.8±7.4	93.4±22.8	279.3±48.5
F1	28.8±13.2	87.3±40.8	286.1±123.4	31.7±10.9	95.8±33.4	313.9±107.4

[a] Data obtained from pages 138-139 (Weeks 0-10 of P generation), 203, and 205 (Weeks 0-14 of the F1generation) in the study report.

4. **Reproductive function**

a. **Estrous cycle length and periodicity:** Estrous cycle length and periodicity were not measured.

b. **Sperm measures:** Sperm enumeration, motility, and morphology were not measured.

4. **Reproductive performance:** Fertility was decreased in the 2700 ppm group at each mating (Table 5). The fertility index was 75-83% at this dose compared to concurrent controls (96-100%) and historical controls (88-100%). However, when fertility was reevaluated in those animals that were not observed to be fertile, most of the animals were proven fertile. In the P generation, only one 2700 ppm female did not prove its fertility. In the F1 generation, one 300 ppm male was not reevaluated due to a technical error; one 900 ppm female, one 2700 ppm male, and one 2700 ppm female did not prove to be fertile. No treatment-related effects were observed on any other reproductive parameters (i.e. mating or gestation indices or on the pre-coital interval or duration of gestation).

TABLE 5. Reproductive performance [a]

Observation	Dose group (ppm)			
	Control	300	900	2700
P Generation (F1a litter)				
Mean (∀SD) precoital interval (days)	2.3±0.85	2.3±0.94	2.6±1.06	2.3±1.11
MALES				
Number mated	24	24	24	24
Number fertile	24	23	23	20
Fertility not determined [b]	0	0	0	0
Intercurrent deaths	0	0	0	0
Male mating index	100	100	100	100
Male fertility index	100	96	96	83
FEMALES				
Number mated	24	24	24	24
Number fertile	24	23	23	20
Fertility not determined [b]	0	0	0	0
Intercurrent deaths	0	0	0	0
Mean (∀SD) gestation interval (days)	22.0±0.20	22.0±0.37	22.0±0.38	22.0±0.60
Number of litters	24	23	22	20
Female mating index	100	100	100	100
Female fertility index	100	96	96	83
Gestation index	100	100	96	100
P Generation (F1b litter)				
Mean (∀SD) precoital interval (days)	2.6±2.43	2.3±1.01	2.3±1.16	2.4±1.14
MALES				
Number mated	24	24	24	24
Number fertile	24	23	24	20
Fertility not determined [b]	0	0	0	0
Intercurrent deaths	0	0	0	0
Male mating index	100	96	100	100
Male fertility index	100	96	100	83
FEMALES				
Number mated	24	24	24	24
Number fertile	24	23	24	20
Fertility not determined [b]	0	0	0	0
Intercurrent deaths	0	1 [c]	0	0
Mean (∀SD) gestation interval (days)	21.9±0.28	21.8±0.49	22.0±0.20	21.7±0.47
Number of litters	24	23	24	20
Female mating index	100	96	100	100
Female fertility index	100	100	100	83
Gestation index	100	100	100	100
F1 Generation				
Mean (∀SD) precoital interval (days)	2.2±1.11	2.4±1.18	2.8±1.11	2.8±2.86
MALES				
Number mated	24	24	24	24
Number fertile	23	21	22	18*
Fertility not determined [b]	0	1	0	0
Intercurrent deaths	1	0	0	0
Male mating index	100	100	96	100
Male fertility index	96	88	92	75

(table continues next page)

TABLE 5. Reproductive performance [a]

Observation	Dose group (ppm)			
	Control	300	900	2700
	FEMALES			
Number mated	24	24	24	24
Number fertile	23	21	22	18
Fertility not determined [b]	0	0	0	0
Intercurrent deaths	1	0	1 [c]	0
Mean (±SD) gestation interval (days)	22.0±0.22	22.0±0.32	22.0±0.49	21.9±0.42
Number of litters	22	21	22	18
Female mating index	100	100	96	100
Female fertility index	96	88	96	75
Gestation index	96	100	100	100

[a] Data obtained from pages 159-163 and 220-223 in the study report.
[b] Fertility was revaluated. The fertility or lack of fertility was confirmed in each animal except one 300 ppm male (due to a technical error).
[c] Animal died while giving birth
* Statistically different from control, $p<0.05$.

6. **Parental postmortem results**

a) **Organ weights**: Organ weights were not measured.

b) **Pathology**

1) **Macroscopic examination**: There were no treatment-related effects observed grossly in the P or F1 parents.

2) **Microscopic examination**: There were no treatment-related effects observed microscopically in the P or F1 parents.

7. **Clinical chemistry**: There were no treatment-related effects observed in clinical chemistry. Decreases ($p\leq0.05$) in alkaline phosphatase in all treated groups relative to controls were noted at Day 141 (↓19-24%). These decreases were unrelated to dose and were not biologically significant. Decreased ($p\leq0.05$) alanine aminotransferase was observed at 300 and 900 ppm, but this effect was unrelated to dose. Enzyme levels in treated groups were similar to controls at Day 152. In the F1 2700 ppm females, minor decreases ($p\leq0.01$) in creatinine (↓8-13%) on Days 141 and 152, as well as a minor increase ($p\leq0.01$) in chlorine (↑3%) on Day 141, were noted.

B. OFFSPRING

1. **Viability and clinical signs:** Mean litter size and viability results from pups during lactation are summarized in Table 6. Decreased ($p \leq 0.01$) mean litter size was observed at Days 0 (↓34%) and 4 before culling (↓30%) in the F1a litter. On Days 0 and 4 (before culling), mean litter size was also decreased (NS) in the F1b (↓9-14%) and F2 (↓16-17%) litters. There were no treatment-related effects on the number of pups born dead, or deaths during Days 0-4 and 5-21; sex ratio; or the live birth, viability, and lactation indices in the F1a, F1b, or F2 litters. No treatment-related clinical signs were reported.

TABLE 6. Litter parameters for F1 and F2 generations [a]

Observation	Dose group (ppm)			
	Control	300	900	2700
F1a Generation				
Mean implantation sites	NR	NR	NR	NR
Number born live	338	306	305	185
Number born dead	5	7	4	2
Sex ratio day 0 (% %)	47.9	52.3	53.8	45.4
# Deaths days 0-4 (%)	9	4	4	4
# Deaths days 4-21 (%)	2	0	0	1
Mean litter size Day 0	14.1±3.72	13.3±3.69	13.9±2.49	9.3±4.18** (↓34)
Day 4 [b]	12.8±3.83	12.8±3.59	13.3±2.29	8.9±3.93** (↓30)
Day 4 [c]	7.6±1.14	7.6±1.56	7.9±0.43	6.8±2.07
Day 7	7.5±1.38	7.6±1.56	7.9±0.43	6.7±2.23
Day 14	7.5±1.38	7.6±1.56	7.9±0.43	6.7±2.23
Day 21	7.5±1.38	7.6±1.56	7.9±0.43	6.7±2.23
Live birth index	99	98	99	99
Viability index	91	96	96	96
Lactation index	98	100	100	99
F1b Generation				
Mean implantation sites	NR	NR	NR	NR
Number born live	354	349*	358**	267
Number born dead	21	9*	6**	17
Sex ratio day 0 (% %)	47.7	51.9	52.2	49.8
# Deaths days 0-4 (%)	5	6	4	9
# Deaths days 4-21 (%)	1	1	0	0
Mean litter size Day 0	14.8±2.69	15.2±3.07	14.9±3.05	13.4±2.68 (↓9)
Day 4 [b]	14.0±2.77	14.3±4.13	14.3±3.08	12.1±2.47 (↓14)
Day 4 [c]	8.0±0.20	7.7±1.67	7.9±0.28	7.9±0.22
Day 7	8.0±0.20	7.6±1.67	7.9±0.28	7.9±0.22
Day 14	7.9±0.28	7.6±1.67	7.9±0.28	7.9±0.22
Day 21	7.9±0.28	7.6±1.67	7.9±0.28	7.9±0.22
Live birth index	94	97	98	94
Viability index	95	94	96	91
Lactation index	99	99	100	100

(table continues next page)

TABLE 6. Litter parameters for F1 and F2 generations [a]

Observation	Dose group (ppm)			
	Control	300	900	2700
F2 Generation				
Mean implantation sites	NR	NR	NR	NR
Number born live	266	235	245	181
Number born dead	5	8	10	3
Sex ratio day 0 (% %)	48.5	55.3	46.1	45.9
# Deaths days 0-4 (%)	5	6	13	4
# Deaths days 4-21 (%)	0	2	6	2
Mean litter size Day 0	12.1±3.25	11.2±4.56	11.1±4.40	10.1±3.61 (↓17)
Day 4 [b]	11.5±3.58	10.6±4.44	9.6±4.26	9.7±3.31 (↓16)
Day 4 [c]	7.5±1.34	7.0±2.13	7.0±2.27	7.3±1.24
Day 7	7.5±1.34	6.9±2.30	6.6±2.68	7.3±1.24
Day 14	7.5±1.34	6.9±2.30	6.6±2.68	7.2±1.22
Day 21	7.5±1.34	6.9±2.30	6.6±2.68	7.2±1.22
Live birth index	98	97	96	98
Viability index	95	94	87	96
Lactation index	100	98	94	98

[a] Data obtained from pages 161, 162, 164-169, 221, and 224-226 in the study report.
[b] Before standardization (culling)
[c] After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$
NR Not reported

2. **Body weight:** Mean pup body weight data are presented in Table 7. Decreased ($p\leq0.01$) body weights were observed in the F1a generation at 2700 ppm on PND 14 and 21 in males, females, and combined males and females (↓13-18%). Decreased ($p\leq0.05$) body weights were observed in the F2 generation at 2700 ppm on PND 7, 14, and 21 in males, females, and combined males and females (↓12-26%). Body weights were similar to controls in the 300 and 900 ppm groups.

TABLE 7. Mean (±SD) Litter and pup weights (g) [a]

Lactation day	Dose group (ppm) 0	300	900	2700	0	300	900	2700
	F1a Litters				F2 Litters			
0	5.7±0.35	5.8±0.42	5.8±0.36	6.1±0.67*	6.0±0.34	6.0±0.56	6.1±0.44	6.0±0.39
4[b]	8.8±1.46	9.3±1.20	9.1±1.08	9.3±2.28	8.9±0.97	9.0±1.33	9.4±1.76	8.8±1.56
4[c]	8.9±1.44	9.3±1.18	9.2±1.13	9.3±2.26	9.0±0.99	9.1±1.35	9.4±1.74	8.8±1.51
7	14.7±2.39	15.5±1.65	15.5±1.63	14.0±3.37	14.8±1.90	14.8±2.61	15.4±1.73	12.9±2.30* (↓13)
14	32.4±4.06	33.8±2.55	33.3±3.33	28.0±5.82** (↓14)	31.4±4.53	30.6±5.39	31.3±2.79	24.9±3.32** (↓21)
21	53.5±5.86	56.4±5.23	55.1±3.72	44.4±8.67** (↓17)	50.8±6.53	49.8±7.22	50.4±4.46	38.0±4.96** (↓25)
	F1a Pups – male				F2 Pups – male			
1	5.9±0.34	5.9±0.37	6.0±0.38	6.2±0.69	6.2±0.34	6.2±0.58	6.2±0.45	6.2±0.40
4[b]	9.0±1.49	9.3±1.10	9.3±1.09	9.4±2.42	9.2±1.06	9.2±1.43	9.4±1.84	9.1±1.56
4[c]	9.1±1.50	9.4±1.09	9.4±1.15	9.4±2.41	9.2±1.05	9.4±1.46	9.5±1.81	9.1±1.53
7	15.1±2.60	15.6±1.59	15.8±1.68	14.0±3.44	15.0±1.94	15.1±2.93	15.3±1.86	13.2±2.31* (↓12)
14	32.9±4.86	33.9±2.06	33.8±3.36	28.0±5.97** (↓15)	31.8±4.67	31.4±6.13	31.4±2.99	25.3±3.52** (↓20)
21	55.0±7.24	57.1±3.79	56.6±4.07	44.9±9.19** (↓18)	51.9±6.87	51.2±8.97	51.0±4.80	38.8±5.19** (↓25)
	F1a Pups – female				F2 Pups – female			
1	5.5±0.32	5.7±0.45	5.7±0.34	5.9±0.67*	5.9±0.39	5.8±0.41	5.9±0.46	5.8±0.42
4[b]	8.5±1.29	9.0±1.18	8.9±1.09	9.1±2.18	8.9±0.81	8.8±1.28	9.3±1.78	8.6±1.56
4[c]	8.5±1.26	9.1±1.17	9.0±1.12	9.2±2.16	9.0±0.86	8.9±1.27	9.3±1.79	8.6±1.51
7	14.1±2.22	15.2±1.67	15.2±1.67	13.7±3.18	14.9±1.44	14.6±2.42	15.4±1.61	12.7±2.30** (↓15)
14	31.7±3.20	33.4±2.77	32.8±3.36	27.6±5.40** (↓13)	31.7±3.07	30.2±4.92	31.4±2.45	24.6±3.23** (↓22)
21	51.9±4.49	54.9±5.52	53.3±3.60	43.3±7.87** (↓17)	50.6±4.44	48.7±6.08	49.8±4.57	37.4±4.84** (↓26)

[a] Data obtained from pages 170, 171, 227, and 228 in the study report.
[b] Before standardization (culling)
[c] After standardization (culling)
**Statistically different from control, $p<0.01$

5. **Sexual maturation (F1):** Sexual maturation landmarks were not measured.

6. **Offspring postmortem results**

a) **Organ weights:** Organ weights were not measured.

b) **Pathology**

1) **Macroscopic examination:** No treatment-related effect was observed grossly.

2) **Microscopic examination:** No treatment-related effect was observed during microscopic examination.

7. **Pup physical development and reflex data:** At 2700 ppm in the F1a, F1b, and F2 generations, fewer ($p \leq 0.01$) pups were noted with pinna unfolding (80-84% treated vs 91-98% controls), auditory canal opening (84-91% vs 97-99%), and eye opening (77-81% vs 94-100%; Table 8). In addition, fewer ($p \leq 0.01$) pups demonstrated the gripping reflex at 2700 ppm in the F1a generation (95% treated vs 100% controls), but the frequency of gripping-reflex demonstration was similar to controls in the F1b and F2 generations. Acoustic startle and pupil constriction responses were similar in the treated groups to controls.

TABLE 8. Number of pups reaching criteria/number of pups tested (%) [a]

Parameter	Dose group (ppm)			
	0	300	900	2700
F1a Generation				
Pinna unfolding	290/307 (94)	281/295 (95)	274/292 (94)	143/178** (80)
Auditory canal opening	175/180 (97)	171/174 (98)	170/174 (98)	113/134** (84)
Eye opening	177/180 (98)	166/174 (95)	166/174 (95)	96/134** (72)
Gripping reflex	180/180 (100)	174/174 (100)	174/174 (100)	127/134** (95)
Acoustic startle	179/179 (100)	174/174 (100)	174/174 (100)	134/134 (100)
Pupil constriction	179/179 (100)	174/174 (100)	174/174 (100)	134/134 (100)
F1b Generation				
Pinna unfolding	306/336 (91)	291/329 (88)	336/342** (98)	203/242** (84)
Auditory canal opening	185/190 (97)	165/174 (95)	188/190 (99)	143/159** (90)
Eye opening	179/190 (94)	160/174 (92)	190/190** (100)	129/159** (81)
Gripping reflex	190/190 (100)	174/174 (100)	190/190 (100)	158/159 (99)
Acoustic startle	190/190 (100)	173/174 (99)	190/190 (100)	159/159 (100)
Pupil constriction	190/190 (100)	173/174 (99)	190/190 (100)	159/159 (100)
F2 Generation				
Pinna unfolding	248/254 (98)	204/222** (92)	202/212 (95)	141/174** (81)
Auditory canal opening	164/166 (99)	141/145 (97)	142/145 (98)	118/130** (91)
Eye opening	156/166 (94)	136/145 (94)	139/145 (96)	100/130** (77)
Gripping reflex	166/166 (100)	144/145 (99)	145/145 (100)	130/130 (100)
Acoustic startle	166/166 (100)	145/145 (100)	145/145 (100)	130/130 (100)
Pupil constriction	166/166 (100)	145/145 (100)	145/145 (100)	130/130 (100)

[a] Data obtained from pages 178-179 and 231 in the study report.

* Statistically different from control, $p<0.05$.

** Statistically different from control, $p<0.01$.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** It was concluded that the LOAEL for parental toxicity was 900 ppm based on decreased body weight gains of the P females during lactation of the F1a pups. At 2700 ppm, the following signs of toxicity were noted: transient increased incidence of tremor and hypersensitivity during the lactating period when food consumption was increased; decreased food consumption and body weight gains in both sexes; and decreased serum creatinine levels in the F1 females. The LOAEL for offspring toxicity was 2700 ppm, based on decreased fertility within the scheduled mating period, decreased number of delivered pups/dam, and decreased growth and development.

B. **REVIEWER COMMENTS**

1. **PARENTAL ANIMALS:** No treatment-related adverse effect was observed in the parents on mortality, food consumption, mating or gestation indices, gestation duration, clinical chemistry, or on gross or histological pathology.

   At 900 ppm in the P generation dams, mean body weight gain during lactation was decreased in the first litter by 52% ($p \leq 0.01$) and in the second litter by 72% (NS); however, these decreases were not accompanied by decreased body weight. Reductions in body weight gain were not observed in the F1 generation dams, primarily because the 900 ppm group's body weight gain was significantly ($p \leq 0.01$) higher than controls during Days 14-21. An explanation for this discrepancy was not provided.

   Decreased ($p \leq 0.05$) body weights and body weight gains were observed in the 2700 ppm females of the P and F1generations (↓5-24%), generally throughout the study hut usually most severe during the lactation periods. Decreased ($p \leq 0.05$) body weight gains were observed during pre-mating (↓11% in P generation only), gestation (↓10-23%), and lactation (↓143-238%). Although food efficiency was not reported, the effect on body weight gain without a clear effect on food consumption suggests that food efficiency was decreased.

   Treatment-related clinical signs observed in the P generation included tremor in the 2700 ppm females during Weeks 26-27 (5-6 rats/week) and during LD 4-21 (3-20 rats/day), and hypersensitivity during lactation of the F1h litter on Days 4-21 (3-4 rats/day). Tremor was also observed in the F1 dams during lactation on Days 3-24 (4-18 rats/day).

   In the F1 2700 ppm females, decreases ($p \leq 0.01$) in creatinine (↓8-13%) on Days 141 and 152 were considered minor and not biologically relevant.

   The LOAEL for parental toxicity is 2700 ppm (approximately equivalent to 254.6 mg/kg/day in males and 279.3 mg/kg/day in females), based on decreased body weights and body weight gains of the P and F1 generations, and increased incidences of tremor and hypersensitivity. The NOAEL is 900 ppm (approximately equivalent to 86.4mg/kg/day in males and 93.4 mg/kg/day in females).

2. **REPRODUCTIVE PERFORMANCE:** No treatment-related effect was observed on male or female mating indices. Fertility was decreased in the 2700 ppm group at each mating. The fertility index was 75-83% at this dose compared to concurrent controls (96-100%) and historical controls (88-100%). However, when fertility was reevaluated in those animals that were not observed to be fertile, most of the animals were proven fertile. In the P generation, only one 2700 ppm female did not prove its fertility. In the F1 generation, one 300 ppm male was not reevaluated due to a technical error; one 900 ppm female, one 2700 ppm male, and one 2700 ppm female did not prove to be fertile. During re-evaluation, all animals not proven fertile were mated with proven fertile controls. This methodology may confound the interpretation of the compound's effect on fertility as only one of the partners was subjected to treatment.

The LOAEL for reproductive performance is 2700 ppm (equivalent to 254.6/279.3 mg/kg/day in males/females), based on decreased fertility index. The NOAEL is 900 ppm (equivalent to 86.4/93.4 mg/kg/day in males/females).

3. **OFFSPRING:** No treatment-related effect was observed on number of pups born dead; live birth, viability, or lactation indices; sex ratio; acoustic startle and pupil constriction responses; or gross or histological pathology

At 2700 ppm, decreased ($p \leq 0.01$) mean litter size was observed at Days 0 (↓34%) and 4 before culling (↓30%) in the F1a litter. On Days 0 and 4 (before culling), mean litter size was also decreased (NS) in the F1b (↓9-14%) and F2 (↓16-17%) litters.

At 2700 ppm, decreased ($p \leq 0.01$) body weights were observed in the F1a generation on PND 14 and 21 in males, females, and combined males and females (↓13-18%). Decreased ($p \leq 0.05$) body weights were observed in the F2 generation at 2700 ppm on PND 7, 14, and 21 in males, females, and combined males and females (↓12-26%). These decreased weights may be secondary to decreased maternal weights.

At 2700 ppm in the F1a-, F1b-, and F2 generations, fewer ($p \leq 0.01$) pups were noted with pinna unfolding (80-84% treated vs 91-98% controls), auditory canal opening (84-91% vs 97-99%), and eye opening (77-81% vs 94-100%). In addition, fewer ($p \leq 0.05$) pups demonstrated the gripping reflex at 2700 ppm in the F1a generation (95% treated vs 100% controls), but the frequency was similar to controls in the F1b and F2 generations. The delay in developmental landmarks may have been related to the decreased body weight and body weight gain.

The LOAEL for offspring toxicity is 2700 ppm (equivalent to 254.6/279.3 mg/kg/day in males/females), based on decreased mean litter size, decreased body weight, and delayed development. The NOAEL is 900 ppm (equivalent to 86.4/93.4 mg/kg/day in males/females).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

C. **STUDY DEFICIENCIES**: The following deficiencies were observed, but were considered minor:

- Dates were not provided specifying when the historical control data were obtained.
- Estrous cycle length and periodicity were not tested.
- No sperm measures were performed.
- Organ weights were not measured in parental or pup animals.
- Sexual maturation landmarks were not evaluated.

DATA EVALUATION RECORD

CHLORMEQUAT CHLORIDE

Study Type: §83-1b, Chronic Toxicity Study in Dogs

Work Assignment No. 3-1-104 C (MRID 46715201)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

78

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** Karlyn
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 6/27/06
Work Assignment Manager: P.V. Shah, Ph. D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/12/06

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity in Dogs (feeding); OPPTS 870.4100b [§83-1b]; OECD 452.

PC CODE: 018101
TXR#: 0054020

DP BARCODE: D325193

TEST MATERIAL (PURITY): Chlormequat chloride (67.4% a.i., dose levels adjusted for purity)

SYNONYMS: (2-Chloroethyl)trimethylammonium chloride

CITATION: Mellert, W. (1993) Report on the study of the toxicity of chlormequat-chloride in beagle dogs: administration via the diet over 12 months. Department of Toxicology, BASF Aktiengesellschaft, Ludwigshafen/Rhein, FRG. Laboratory Report Nos.: 33D0580/87120, October 1, 1993. MRID 46715201. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 46715201), Chlormequat chloride (67.4% a.i., dose levels adjusted for purity, Batch #: 82-0767) was administered to 5 beagle dogs/sex/dose in the diet for 52 weeks at doses of 0, 150, 300, or 1000 ppm (approximately equivalent to 0, 5, 10, and 32 mg/kg/day).

There were no treatment-related adverse effects observed on food consumption, food efficiency, ophthalmoscopic examination, brain cholinesterase, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, or histopathology.

At 300 ppm and above, incidences of salivation were increased in the males (3-5 treated vs. 0 controls) and females (2-5 treated vs. 0 controls). Diarrhea was observed in the males at these doses (2-5 treated vs. 0 controls), and vomiting was noted in a single female at 300 ppm. At 300 ppm, cumulative body weight gain was decreased (5-36%; not significant [NS]) in males beginning on Day 21 and lasting until Day 231 where it had become comparable to controls.

At 1000 ppm, treatment-related deaths were observed in one male on Day 42 and one female on Day 20. Histopathological examination in these animals revealed moderate or marked serous

lung edema. Also, marked depletion of thymic lymphocytes was noted in the male and multiple thymic hemorrhages were noted in the female. All other animals survived until scheduled sacrifice. Clinical signs of neurotoxicity were observed in the animals that died, including: emaciation, apathy, staggering gait, lateral position, saltatory spasm, and vomitus in the male and unsteady gait in the female. Additionally at 1000 ppm, cumulative body weight gain was decreased (90-133% [NS]) in males mostly due to one animal which died on Day 42. After the death of this animal, body weight gain was still decreased (14-31%; [NS]) during Days 42-364.

The LOAEL is 300 ppm (approximately equivalent to 10 mg/kg/day), based upon salivation (both sexes), vomiting (in females), and diarrhea (in males), and decreases in body weight gains in males. The NOAEL is 150 ppm (approximately equivalent to 5 mg/kg/day).

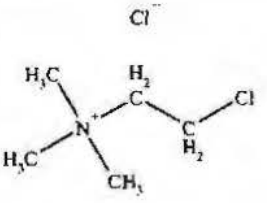
This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

COMPLIANCE - Signed and dated GLP compliance, Data Confidentiality, Quality Assurance, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Chlormequat Chloride

Description:	Brown-yellowish liquid
Batch #:	82-0767
Purity:	67.4% a.i., dose levels adjusted for purity
Compound stability:	Stable in diet for up to 24 hours at room temperature
CAS # of TGAI:	999-81-5
Structure:	

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	Approximately 5-7 months; 7.6-9.8 kg males, 6.4-9.5 kg females	
Source:	BASF (Rhein, FRG)	
Housing:	Individually in pens with 24-hour indoor and outdoor access during test substance administration	
Diet:	KLIBA laboratory diet 335; 700 g/day for 3h/day made into a paste using 1:1 powdered diet: water (Klingentalmuhle AG, Kaiseraugst, Switzerland); except during fasting prior to blood and urine collection.	
Water:	Demineralized water adjusted with drinking water, *ad libitum*, except during urine collection where 500 mL was available	
Environmental conditions:	**Temperature:**	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Photoperiod:	Natural day/light rhythm, with additional artificial light supplied as necessary
Acclimation period:	7 days	

B. STUDY DESIGN:

1. In life dates: Start: 4/03/89 End: 4/11/90

2. Animal assignment: The dogs were stratified by pre-exposure body weight and then randomly assigned to the test groups shown in Table1.

Table 1. Study design [a]			
Test Group	Dose (ppm)	Achieved Intake (mg/kg/day)	# of Animals (M/F)
Control	0	0	5/5
Low	150	5	5/5
Mid	300	10	5/5
High	1000	32	5/5

a Data were obtained from pages 15, 16, and 25 of the study report

3. **Dose selection rationale:** It was stated that the dose-selection was based on the results of two range-finding studies (270-1200 ppm), a 3-month dietary toxicity study (20-180 ppm), a 12-month dietary toxicity study (300 ppm), and a 2-year dietary toxicity study (100-1000 ppm) in dogs. At 1200 ppm, diarrhea and lacrimation, high-stepping gait, unsteady gait and weakness of the hind limbs, and moderate to severe salivation were observed. At 1000 ppm, mortality of one male and one female, and pronounced salivation, diarrhea, unsteady gait, and muscle weakness of the hind limbs were observed. At ≥ 300 < 1000 ppm, slight to moderate salivation was observed. There were no effects on hematology, clinical chemistry, organ weights, and gross or histopathological examinations. Therefore, 1000 ppm was chosen as the high-dose based on these results, and the remaining dose levels were chosen in an attempt to provide dose-response data and a NOAEL.

4. **Diet preparation and analysis:** Test diets were prepared immediately before administration by mixing 350 grams of food pellets with 350 grams of test substance/drinking water solution to form a paste. Test diet concentrations were adjusted for purity as reported in a multigenerational reproductive study in rats (MRID 46715206), reviewed concurrently with this study. In this mixing process, the test substance was diluted with drinking water to the desired concentration and stirred with an apparatus for approximately 10 minutes. The dogs received the paste mixture until the day before necropsy. It was stated that homogeneity of the test compound was demonstrated, however, no homogeneity analyses were provided as part of the current study. In the aforementioned reproductive study (MRID 46715206), six samples each from 500 and 4500 ppm dietary formulations were analyzed to verify homogeneity prior to animal treatment. The stability of the test compound in drinking water was demonstrated for 34 days at room temperature. The stability of the test compound in the food paste mixture was also demonstrated at 270 and 539 ppm for up to 24 hours at room temperature. Concentration analyses were performed on all dietary concentrations prior to the start of the study, and at about 3-month intervals thereafter.

Results:

Stability of the test substance in the food paste mixture (% of time 0): 99.4-101.3%

Homogeneity (% coefficient of variation): 1.8-2.8%

Concentration (% of nominal): 87.4-103.3% (except for the 300 ppm diet on 10/12/89, which measured 78.8% of nominal)

The analytical data indicated that the variation between nominal and actual dosage to the animals

was acceptable.

5. **Statistics:** The following statistical procedures were used:

Parameter	Statistical Test
Food consumption, test substance intake.	Descriptive statistics (means and standard deviations).
Body weight, body weight gain, clinical chemistry and hematology (except differential blood count).	One way ANOVA using Kruskal-Wallis-H-test. If $p \leq 0.05$, then a Mann-Whitney U-test was performed for a pairwise comparison of each dose group with the control group.
Urinalysis	Chi ^{2} test in 2 x 2 contingency tables
Organ weights	A non-parametric one way ANOVA using the Kruskal-Wallis-H test. If $p \leq 0.05$, then a Wilcoxon-U test was performed for a pairwise comparison of each dose group with the control group.

The statistical methods were considered appropriate.

C. METHODS:

1a. Clinical Observations: Animals were observed twice daily (Mondays-Fridays) for mortality and moribundity. On weekends and holidays, animals were observed once daily. Daily cage-side examinations for clinical signs of toxicity were also performed. Prior to treatment, all animals were vaccinated and subjected to deworming.

1b. Neurological evaluations: Dogs were initially examined 4 times daily and then in weekly intervals starting on study Day 91 for signs of diarrhea, salivation, lacrimation, or impairment of motor function. Pupillar reflex was examined at the same time that body weight was determined. Further examinations of pupillar reflex, as well as pain reflex and hearing tests were performed if there was impairment of coordination or movement.

2. Body weight: All animals were weighed prior to the study, weekly during the study, and at termination. Cumulative body weight gains were calculated weekly throughout the study.

3. Food consumption, food efficiency, and compound intake: Food consumption (g/animal/day) was measured daily prior to and throughout the study. Food efficiency (%) was calculated at 4-week intervals on the basis of body weight gain and food consumption values. Test substance intake (mg/kg bw/day) was calculated daily throughout the study using the food consumption, body weight, and nominal dietary concentration data.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were conducted on all dogs pre-exposure and all surviving control and 1000 ppm dogs prior to termination.

5. Hematology and clinical chemistry: Blood samples for hematology and clinical chemistry analyses were collected from the vena cephalica antebrachii of all animals (fasted) prior to treatment, on Days 94, 184, and 360. The following CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time)		
X	(Partial thromboplastin time)		
	(Prothrombin time)		

* Recommended for chronic studies based on Guideline 870.4100.

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
-	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
X	Serum cholinesterase (ChE)	X	Triglycerides
X	Erythrocyte cholinesterase (EChE)	-	Serum protein electrophoresis
X	Brain cholinesterase (BChE) +		
-	Creatine phosphokinase		
-	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
-	Glutamate dehydrogenase		
-	Sorbitol dehydrogenase*		

* Recommended for chronic studies based on Guideline 870.4100.
+ Brain cholinesterase only determined at terminal sacrifice.
- Not examined

6. **Urinalysis:** Urine samples were collected from all animals (fasted) prior to treatment, on Days 92, 183, and 358. Samples were collected overnight from individual animals in metabolism cages without food (500 mL of water was available). The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood*
X	Sediment (microscopic)	X	Nitrite
X	Protein*	X	Urobilinogen

* Recommended for chronic studies based on Guideline 870.4100.

7. **Sacrifice and pathology:** At study termination, all surviving animals were fasted, weighed, anesthetized, killed via exsanguination, and subjected to a gross pathological and histopathological examination. Also on Day 365, brain cholinesterase measurements were obtained using the Ellman method. The following CHECKED (X) tissues were collected and examined microscopically in all animals. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
-	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	X	Heart*+	X	Periph.nerve(sciatic nerve)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	-	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	XX	Thyroids*
XX	Liver*+	XX	Testes*+		OTHER
X	Gall bladder*	-	Epididymides*+	X	Bone (sternum and femur)
X	Pancreas*	X	Prostate*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+	X	Skin*
X	Trachea*	X	Uterus*+	X	All gross lesions and masses*
X	Lung*++	X	Mammary gland*		
-	Nose*	X	Vagina		
-	Pharynx*				
-	Larynx*				

* Required for chronic studies based on Guideline 870.4100.
\+ Organ weight required in chronic studies.
++ Organ weight required if inhalation route.
\- Not examined

Samples to be examined microscopically were fixed in 4% formaldehyde solution, prepared routinely, and stained with hematoxylin and eosin. Additionally, Oil red O stain was used for the liver. Findings were reported as present or assigned a severity grade.

II. RESULTS:

A. OBSERVATIONS:

1. **Mortality:** At 1000 ppm, treatment-related deaths were observed in one male (# 273:8) on Day 42 and one female (# 274:8) on Day 20. Histopathological examination in these animals revealed moderate or marked serous lung edema. Also, marked depletion of thymic lymphocytes was noted in the male and multiple thymic hemorrhages were noted in the female. All other animals survived until scheduled sacrifice.

2. **Clinical/Neurological signs of toxicity:** At ≥ 300 ppm, incidences of salivation, beginning at week 1 and lasting up to week 53, were increased in the males (3-5 treated animals vs. 0 controls) and females (2-5 treated animals vs. 0 controls). Diarrhea, first observed at week 1 and

lasting up to week 6, occurred 4-9 times in the males (2-5 treated animals vs. 0 controls), and occurred 1-2 times in the females (3 treated animals vs. 0 controls); at week 30, one occurrence of vomiting was noted in a single female at 300 ppm.

Additionally at 1000 ppm, clinical signs of neurotoxicity were observed in the animals that died, including: emaciation, apathy, staggering gait, lateral position, saltatory spasm, and vomitus in male # 273:8; and unsteady gait in female # 274:8.

Table 2. Clinical/neurological signs of toxicity, # animals (# of occurrences)/52 weeks, in dogs treated with Chlormequat chloride in the diet for up to 1 year [a]

Clinical sign	Dose (ppm)			
	0	150	300	1000
Males				
Salivation	0	0	3 (3)	5 (5)
Diarrhea	0	0	2 (4-7)	5 (6-9)
Staggering gait, emaciation, apathy	0	0	0	1 (1[b])
Saltatory spasm, lateral position	0	0	0	1 (1[b])
Females				
Salivation	0	0	2 (2)	5 (5)
Diarrhea	0	0	0	3 (1-2)
Vomiting	0	0	1 (1)	0
Unsteady gait	0	0	0	1 (1[c])

a Data were obtained from page 200, and pages 547-548 of the study report.
b Animal (# 273:8) died on Day 42.
c Animal (# 274:8) died on Day 20.

B. BODY WEIGHT AND WEIGHT GAIN: Body weight and body weight gain data are presented in Table 3. Slightly decreased (↓ 3-4%; not significant [NS]) body weights were observed in the 1000 ppm males beginning on Day 133 but were very minor and sporadic. Body weights in the remaining treatment groups were comparable to controls.

At 300 ppm, cumulative body weight gain was decreased (↓ 5-36%; [NS]) in males beginning on Day 21 and lasting until Day 231 where it had become comparable to controls. At 1000 ppm, cumulative body weight gain was decreased (↓ 90-133% [NS]) in males mostly due to animal # 273:8 which died on Day 42. However, after the death of this animal, body weight gain was still decreased (↓ 14-31%; [NS]) during Days 42-364.

The decreases in body weight gains observed in all treated females were highly variable and followed no apparent dose-response relationship. Therefore, this effect in females was considered incidental and unrelated to treatment.

Table 3. Mean (±SD) body weights and cumulative body weight gain (kg) at selected times in dogs treated with Chlormequat chloride in the diet for up to 1 year [a]

Days on Study	Dose (ppm)			
	0	150	300	1000
Males				
0	8.6±0.7	8.7±0.8	8.8±0.7	8.8±0.7
91	11.3±0.4	11.4±0.7	11.1±0.3	11.0±0.8
133	12.1±0.4	12.1±0.9	11.7±0.3	11.7±0.9 (↓3)
364	12.2±0.7	12.1±0.8	12.3±0.6	11.7±1.1 (↓4)
BWG: 0-91	2.6±0.7	2.7±0.4	2.3±0.7 (↓12)	2.1±0.7 (↓19)
BWG: 0-182	3.8±1.1	3.6±0.8	3.5±0.9 (↓8)	3.2±1.1 (↓16)
BWG: 0-273	3.4±1.4	3.2±0.8	3.4±1.0	2.8±1.0 (↓18)
BWG: 0-364	3.6±1.3	3.4±0.7	3.5±1.0	2.8±1.0 (↓22)
Females				
0	7.8±1.0	8.0±0.9	7.9±1.1	8.1±1.0
91	10.4±1.0	10.2±0.7	10.1±0.9	10.5±1.3
364	11.6±1.8	10.4±0.8	10.8±1.7	11.7±1.4
BWG: 0-91	2.6±0.3	2.2±0.5	2.3±0.4	2.3±0.2
BWG: 0-182	3.7±1.2	2.8±0.3	3.1±0.8	3.5±0.4
BWG: 0-273	3.6±0.6	2.2±0.7 (↓39)	2.5±1.1 (↓31)	3.3±0.8 (↓8)
BWG: 0-364	3.9±1.0	2.4±0.8 (↓38)	3.0±1.2 (↓23)	3.5±0.7 (↓10)

a Data obtained from Tables 95-122 on pages 156-183 of the study report; n=4-5. Percent differences from controls (calculated by reviewers) are included in parentheses.

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND COMPOUND INTAKE:

1. Food consumption: At 1000 ppm, food consumption was decreased (↓ 4-20%; [NS]) in both sexes due to the two animals that died prematurely in this treatment group. After the death of these animals, food consumption in the treated males was comparable to controls throughout the remainder of the study, and food consumption in females was comparable to controls through Day 91 and varied in all dose groups (including controls) thereafter.

2. Food efficiency: Food efficiency data were varied throughout the groups but it appeared there were no treatment-related effects.

3. Compound intake: Mean test material intake values for the overall study are reported in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION: No abnormal changes were observed in either sex during the pre-exposure or Week 52 examinations.

E. BLOOD ANALYSES:

1. **Hematology:** There were no treatment-related effects on hematology.

2. **Clinical chemistry:** In the 1000 ppm males, increased cholesterol levels were observed throughout treatment (↑36-39%; $p \leq 0.01$); however, cholesterol levels were increased (↑19%; $p \leq 0.05$) prior to treatment, so this effect was not believed to be treatment-related. All changes observed on clinical chemistry were considered minor, transient, incidental, and/or unrelated to dose.

F. BRAIN CHOLINESTERASE ACTIVITY: Brain cholinesterase activity of treated groups was comparable to controls at study termination. Data are reported in Tables 185 and 190 on pages 246 and 251 of the study report.

G. URINALYSIS: There were no treatment-related effects on urinalysis. On Day 183, there was more than trace levels of blood in the urine of 3/4 females treated at 1000 ppm. This effect was not present at 12 months, therefore it was considered transient and not related to treatment.

H. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** No treatment-related effects on organ weights were observed.

2. **Gross pathology:** There were no abnormal macroscopic findings.

3. **Microscopic pathology:** All microscopic findings were unrelated to dose in incidence and/or severity.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS CONCLUSIONS: The LOAEL was 300 ppm based on effects observed on the nervous system (salivation, vomiting, and diarrhea), as well as the two deaths observed at 1000 ppm.

B. REVIEWER COMMENTS: No treatment-related adverse effects were observed on food consumption, food efficiency, brain cholinesterase, ophthalmoscopic examination, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, or histopathology.

At 1000 ppm, treatment-related deaths were observed in one male on Day 42 and one female on Day 20. Histopathological examination in these animals revealed moderate to marked serous lung edema. Also, marked depletion of thymic lymphocytes was noted in the male and multiple thymic hemorrhages were noted in the female. All other animals survived until scheduled sacrifice. Additionally at 1000 ppm, clinical signs of neurotoxicity were observed in the animals that died, including: emaciation, apathy, staggering gait, lateral position, saltatory spasm, and vomitus in the male and unsteady gait in the female. It was stated in the study report that the test compound has been shown to have reversible effects on nicotinic receptors which would explain

the observed impairment of neurological function noted in this DER.

At ≥ 300 ppm, incidences of salivation were increased in the males (3-5 treated vs. 0 controls) and females (2-5 treated vs. 0 controls). Diarrhea was observed in the males at these doses (2-5 treated vs. 0 controls), and vomiting was noted in a single female at 300 ppm.

At 300 ppm, cumulative body weight gain was decreased (↓ 5-36%; [NS]) in males beginning on Day 21 and lasting until Day 231 where it had become comparable to controls. At 1000 ppm, cumulative body weight gain was decreased (↓ 90-133% [NS]) in males mostly due to animal # 273:8 which died on Day 42. After the death of this animal, body weight gain remained decreased (↓ 14-31%; [NS]) during Days 42-364.

The LOAEL is 300 ppm (approximately equivalent to 10 mg/kg/day), based upon salivation (both sexes), vomiting (in females), and diarrhea (in males), and decreases in body weight gains in males. The NOAEL is 150 ppm (approximately equivalent to 5 mg/kg/day).

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

C. **STUDY DEFICIENCIES:** The following deficiencies were observed but do not change the conclusions of this review.

- Reviewers assumed the test substance was adjusted for purity since this was the case in the other study using the same purity and batch # (MRID 46715206, reviewed concurrently) as the current study.
- Temperature, humidity, and air changes were not reported.
- The nose, pharynx, larynx, and epididymides were not examined.
- Heart, spleen, epididymides, and uterine weights were not measured.

EPA Reviewer: Irving Mauer, Ph.D. Date: 07/19/06
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 07/19/06
Toxicology Branch, HED (7509C)
TXR No: 0054020

DATA EVALUATION RECORD

STUDY TYPE: Bacterial systems, *e.g.*, *Salmonella* and *E. coli*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]; OECD 471, 472.

DP BARCODE: D325193

P.C. CODE: 018101 TOX. CHEM. NO.: 191

TEST MATERIAL (PURITY): CL 38,555 (Batch No. AC6779-98A, 66.1% a.i.).

SYNONYMS: Chemically, (2-Chloroethyl)trimethyl ammonium chloride; chlormequat-chloride (common name for ISO); CYCOCEL (trade name).

CITATION: Traul, K.A. (1990). Evaluation of CL 38,555 in a Microbial/Microsome Mutagenicity Test, performed at the Agricultural Research Division of The American Cyanamid Company, Princeton (NJ). Laboratory Report No.: 90-02-001, dated October 24, 1990. MRID 41721610. Unpublished.

SPONSOR/SUBMITTER: American Cyanamid Company, Princeton (NJ).

EXECUTIVE SUMMARY:

In two fully independent and two partial microbial mutagenicity assays (MRID 41721610), cultures of five histidine-deficient (auxotrophic *his*$^-$), strains of *Salmonella typhimuriumn* (TA98, TA100, TA1535, TA1537, and TA1538) and the typtophan-deficient (auxotrophic *try*$^-$) strain (WP2 *uvr*A) of *Escherichia coli* were exposed in the presence and absence of metabolic activation for 48 hours by plate incorporation to the test material (CL 38,555, Batch AC6779-98A, 66.1%) at 5 concentrations ranging from 100 to 5000 ug/plate, as follow: Two initial and repeat "full" assays, *i.e.*, testing the entire battery of six bacterial strains indicated above; as well as testing twice only those strains to determine the reproducibility of suspected spurious positive results obtained in the full trials. In addition to assaying the vehicle, water (negative control), the tester strains were exposed to strain specific mutagens (as positive controls). At harvest, the numbers of revertant colonies (*his*$^+$, *try*$^+$) in tester strains were compared to the number of

colonies in vehicle controls, and the incidences in positive controls versus negative controls were employed to determine the adequacy of the assays. Significant increases in revertant colonies were to be recorded as positive responses, and would represent induced mutagenic events.

Single non-concentration-related positive responses were found in treated TA1537 at 100 *u*g/plate and in TA1538 at 1000 *u*g/plate in the S9-activated portion of the first full trial, as well as in TA1535 at 100 *u*g/plate in the second full trial, both without activation, as well as under S9-activated conditions. These increases were considered non-biological, since they were not observed in two "partial" assays of these three strains. All positive controls responded with marked increases in revertants.

Thus, CL 38,555 (chlormequat-chloride) may be considered non-mutagenic in this battery of bacterial tester strains when assayed up to the limit dose (5000 *u*g/plate ± S9).

This study is classified as **acceptable/guideline** and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test Material: CL 38,555.

Description: Pale, yellow, free-flowing liquid.
Lot/Batch No.: AC6779-98A.
Purity: 66.1% a.i.
Stability and chemical analysis of compound:

Data on stability was stated to have been presented in an "Appendix 3" of the Report but no such attachment was included therein

CAS No. 999-81-5.
Vehicle/Solvent used: Water.
Other comments: None.

2. Control Materials:

Negative: None.
Vehicle/Solvent/Final concentration: Water/Final concentration not provided.

Positive: Non-activated:

MNNG[1]	5/10	*u*g/plate	for TA100/TA1535
2-Nitrofluorene	20	*u*g/plate	for TA1538
9-Aminoacridine	50	*u*g/plate	for TA1537
Other (list): MNNG[1]	10	*u*g/plate	for WP2 *uvr*A.

Activated:

2-Aminoanthracene (2-anthramine) 5 *u*g/plate for all strains.

[1]MNNG = N-methyl-N[1]- nitro-nitrosoguanidine

3. Metabolic Activation: S9, Lot No. MBA R413, obtained from Microbiological Associates, Bethesda (MD), was derived from post-mitochondrial microsomes of Sprague-Dawley males injected intraperitoneally (i.p.) with Aroclor 1254.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other
If other, describe below): None.							

S9-mix composition was prepared according to the methods described by Ames and co-workers (Ames, *et al,* 1975[2]; Maron and Ames, 1983[3]).

4. Test Organisms: *S. typhimurium* strains:

	TA97	x	TA98	x	TA100		TA102
	TA104	x	TA1535	x	TA1537	x	TA1538
List any others: *E. coli WP2 uvr*A.							

Properly maintained: Not stated.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

Sources: *S. typhimurium* strains from Dr. B. N. Ames (U. Cal., Berkeley).
E. coli from Dr. B. Bridges, MCR Cell Unit, University of Sussex (Brighton, ENGLAND).

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[2]Ames, B.N., McCann, J. and Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. Mut. Res. 31:347-364 (1975).

[3]Maron, D. M. and Ames, B. N. Revised methods for the *Salmonella* mutagenicity test. Mut. Res.113: 173-215 (1983).

5. Test Compound Concentrations Used:

Cytotoxicity Test: (Not performed.)

Main Assays: All trials (in triplicate): 500, 1000, 2500, 5000 *u*g/plate ±S9.

B. TEST PERFORMANCE:

According to Ames and co-workers[2,3], agar and minimum medium [Vogel-Bonner Medium E] were layered as a base into plates, followed by a mixture of 0.1 mL of test material, 0.1 mL bacterial cells, and 0.5 mL S9-mix (for activation) or buffer[4] (no activation). Then, 2.0 mL molten top agar with trace amounts of histidine, biotin and tryptophan were added, poured onto the base layers of agar and medium and allowed to solidify. Three replicates per concentration were prepared, and incubated at 37°C. Concurrent positive and vehicle control were run on each test day. After 48 hours, revertant colonies were counted; manually or using an automatic colony counter. The entire "full" assay was performed twice, and two "partial" trials were also performed to substantiate that the positives found in three test strains of the two "full" batteries (TA1535, TA1537, and TA1538) were statistical, non-biological events.

1. Type of *Salmonella* Assay:

x	Standard plate test
	Pre-incubation (_____ minutes)
	"Prival" modification
	Spot test
	Other (describe)

2. Data Evaluation:

The mean values of test article revertants per plate per dose level were compared to the concurrent vehicle control value. If a given concentration point was equal to or greater than twice that of the control, the result was declared "positive", but the following confirmation was required: A

reproducible concentration-related increase in mean numbers of revertants over at least three levels of test material.

II. REPORTED RESULTS:

A. **Chemical Analysis**:

Multiple aqueous solutions of each Trial were presented, as follows:.

Trial	% of Nominal (Range of 5 Concentrations, 1 to 50.0 mg/mL)	Coefficient of Variation %
I	101 - 107 (mean, 104)	3
II	91-115 (mean, 99)	10
III	93-103 (mean, 99)	4
IV	93-106 (mean, 102)	5

(Data from MRID 41721610, pp. 27 to 34, APPENDIX 2).

B. **Preliminary Cytotoxicity Assay**:

There was no preliminary cytotoxicity test.

C. **Mutagenicity Assays**:

In the first of two "full" assays, (Trial I), nonconcentration-related single positive responses (2-fold increases in revertants compared to the vehicle control) were observed in strain TA1537 at 100 *u*g/plate and in strain TA1538 at 1000 *u*g/plate, both in the S9-activated series. Neither positive event occurred in the second trial. In Trial II, a positive was found in TA1535 at 100 ug/plate ±S9. Suspecting these unique positive responses were false positives, these three strains were re-tested at the same concentrations (Trial III).

Neither TA1537 nor TA1538 responded positively to the test article, but a second non-concentration-related positive response was observed in TA1535 at 500 *u*g/plate +S9 and at 100 *u*g/plate -S9. A final trial with TA1535 (IV) demonstrated no positive responses in any test strain.

Therefore, the investigator concluded that the test article was negative for reverse mutation in the conventional battery of bacterial strains. By contrast, the positive controls induced marked increases in revertant colonies both in the presence and the absence of S9-activation in all trials (see ATTACHMENT Tables 1- 4, pp. 14 - 17, MRID 41721610).

III. REVIEWERS' DISCUSSION/CONCLUSIONS:

A . The EPA reviewers agree with the investigator's conclusion that Batch AC 6774-98A of CL 38,555 did not increase the incidence of revertants (*his*$^-$ to *his*$^+$; *try*$^-$ to *try*$^+$) in the conventional battery of bacterial strains when assayed up to 5000 *u*g/plate ± S9.

B. Study Deficiencies: None.

EPA Reviewer: Irving Mauer, Ph.D. Date: 7/19/06
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 07/19/06
Toxicology Branch, HED (7509C)
TOX. NO.: 0054020

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture; gene mutation assay in Chinese hamster ovary (CHO) cells; OPPTS 870.5300 [84-2]; OECD, 476.

DP BAR CODE: D325193 TOX. CHEM. NO.: 191

P.C. CODE: 018101

TEST MATERIAL (PURITY): CL 38,555 (Batch No. AC 6779-98A, 66.1% a.i.)

SYNONYMS: Chemically, (2-chloroethyl) trimethylamonium chloride; chlomequat-chloride (common name suggested to ISO; CYCOCEL®.

CITATION: Traul, K.A. (1990). Evaluation of CL 38,555 in the Mammalian Cell CHO/HGPRT Mutagenicity Test, performed in the Genetic Toxicology Laboratory, Agricultural Research Division of the American Cyanamid Company, Princeton (N.J.). Study No.: 90-05-001, dated November 20, 1990. MRID 41798102. Unpublished.

SPONSOR/SUBMITTER: American Cyanamid Company, Princeton (N.J.).

EXECUTIVE SUMMARY:

In independent mammalian cell forward gene mutation assays (MRID 41798102), cultures of Chinese hamster ovary (CHO) cells, heterozygous at the hypoxanthine-guanine phosphoribosyl transferase locus ($hgprt^+/hgprt^-$), were exposed for 5 hours to CL 38,555 (Batch No. AC 6779-98A, 66.1% a.i., dissolved in sterile water, SW.), in the presence (+S9) and absence (-S9) of an exogenous metabolic activation system (±S9), at six concentrations ranging from 500 to 5000 ug/mL. These concentrations were selected from a previous screening test, which indicated that the test article was not cytotoxic below 3500 ug/mL. In addition to cultures exposed to SW (representing the vehicle, negative, control), additional cultures were treated with the mutagens ethylmethanesulfonate (EMS, 200 ug/mL) and 7, 12-dimethylbenzanthracene (DMBA 3.5 ug/mL) to serve as positive controls for, respectively, the non-activated and S9-activated test series. All cultures were exposed to the substituted purine nitrogenous base, 6-thioguanine (TG). Mutant cells ($tgprt^-/tgprt^-$) have lost their ability to incorporate TG and survive while all other gene variants, homozygates $^{(+/+)}$ and any remaining heterozygotes $^{(+/-)}$, incorporate this altered purine analog and die.

Results obtained in the first S9-activated assay (Trial I), and in both nonactivated (-S9) assays (Trials I and II) demonstrated that CL 38,555 does not induce forward mutations at the HGPRT-locus when exposed to concentrations up to 5000 *u*g/mL. In Trial II, 2500 *u*g/mL alone had a limited positive response, but is not deemed a truly biological response since a linear concentration associated with this increase was absent, and this single increase was not observed in Trial I.

Therefore, CL 38,555 is considered negative for mutation in this test system.

This study is classified as **acceptable/guideline** and satisfies the requirement for *in vitro* gene mutation for FIFRA Test Guideline 84-2 (forward mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test Material: CL 38,555.

Description: Pale yellow liquid.
Lot/Batch No.: AC 6779-98A.
Purity: 66.1% a.i.
Stability of compound: Stability and chemical analyses of dosing solutions are found in APPENDIX 3, pp. 37 to 39 of MRID 41798102.
CAS No.: 999-81-5.
Vehicle/Solvent used: Sterile water (SW).
Other comments: None.

2. Control Materials:

Negative: None.
Solvent/Final concentration: SW/10 *u*L.
Positive: Non-activation (concentrations, solvent): Ethylmethanesulfonate [EMS, 40 *u*g/mL; solvent, dimethyl sulfoxide (DMSO)].

Activation (concentrations, solvent): 7,12-Dimethylbenzanthracene (DMBA), 3.5 *u*g/mL; solvent, DMSO.

3. Metabolic Activation:

S9 (Lot No. R412) purchased from Microbiological Associates, Inc., Bethesda (MD), was derived from hepatic homogenates (S9-fraction) of Aroclor 1254-treated Sprague-Dawley rats (sex not specified).

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other				other		
If other, describe below: None.							

Describe S9-mix composition: [components and concentrations not described.]

4. Test Cells: Mammalian cells in culture.

	Mouse lymphoma L5178Y cells
x	Chinese hamster ovary (CHO) cells (K^1 BH^4 subline)
	V79 cells (Chinese hamster lung fibroblasts)
	Other (list): None.

Properly maintained? Yes.
Periodically checked for Mycoplasma contamination? Yes.
Periodically checked for karyotype stablity? Not stated.
Periodically "cleansed" against high spontaneous background? Not stated.

5. Media: Ham's F12.

6.. Locus Examined:

_____ *thymidine kinase* (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)
_____ trifluorothymidine (TFT)

__x__ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
selection agent: _____ 8-azaguanine (8-AG)
(give concentration) [not reported] 6-thioguanine (6-TG)

_____ Na^+ /K^+ ATPass
selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

7. Test Compound concentrations Used:

a. Range-Finding Test: 500, 1250, 2500, 3500, 4500, 5000 *u*g/mL ±S9.

b. Main Study: 500, 1250, 2500, 3500, 4500, 5000 *u*g/mL ±S9.

B. TEST PERFORMANNCE:

1. Cell Treatment:

a. Cells exposed to test compound, negative/solvent or positive controls for:

__5__ hours (nonactivated) __5__ hours (activated)

b. After washing, cells cultured for __7__ days (expression period) before cell selection:

c. After expression, __10^5__ cells/dish (5 dishes/group) were cultured for __7 to 9__ days in selection medium to determine numbers of mutants; and __100 to 200__ cells/dish (3 dishes/group) were cultured for __7 to 9__ days without selective agent to determine cloning efficiency.

2. Statistical Methods:

The mutation frequencies (MFs) were transformed using the following formula: (1 + mutant frequency) 0.15, followed by regression analysis and ANOVA, to determine any concentration-related effect within the group of samples treated with the test material; the p value was not stated but was assumed to be ≤ 0.05.

3. Evaluation Criteria:

Criteria for acceptable assays and positive responses were presented.

II. REPORTED RESULTS:

A. **Chemical Analyses of Concentrations**:

Six water solutions in the range of 25 to 250 ug/mL assayed from 100% to 109% with a mean of 108% and a coefficient of variation (CV) of 3% (Trial I); and from 106% to 111% with a mean of 109% and a CV of 3% (Trial II). (MRID 41798102, APPENDIX 3, pp. 38 to 40).

B. **Preliminary Cytotoxicity Assay**:

[The range of doses was taken from a previous study in which concentrations up to 35,000 ug/mg were not cytotoxic to CHO cells.] Accordingly, 5000 ug/mL was selected as the highest concentration.

C. **Mutagenicity Assay**:

In Trial I, no treatment level met the criteria for a positive response. In Trial II, however, the average mutant frequencies for the 2500 and 3000 ug/mL concentrations significantly exceeded the concurrent negative control. There was no evidence for a linear concentration-associated effect in either Trial (MRID 41798102, pp.15 to 17, ATTACHMENT Tables 3 to 5). Data for the post-treatment toxicity assays were presented as Tables 1 and 2, pp. 13 and 14 of MRID 41798102. Positive controls showed the appropriate marked increases in mutant colonies.

Therefore, the investigator concluded that CL 38,555 (chlormequat-chloride) was nonmutagenic in CHO cells treated up to 5000 ug/mL.

III. REVIEWERS' DISCUSSION/CONCLUSIONS:

A. The EPA reviewers agree with the conclusions of the investigator that CL 38,555 (chlormequat-chloride, CYCOCEL®) did not induce forward mutation at the HGPRT locus in CHO cells exposed up to the limit dose, 5000 ug/mL.

B. Deficiencies: None.

CHLORMEQUAT-CHLORIDE (AC- 38,555) *In vivo* CHROMOSOME ABERRATION [84-2]

EPA Reviewer: Irving Mauer, Ph.D. Date: 07/19/06
Review Section 3, Toxicology Branch (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 07/19/06
Toxicology Branch (7509C)
TXR No.: 0054020

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics [chromosome aberrations assay] in rats; OPPTS 870.5385 [84-2] OECD 475

DP BAR CODE: D325193 TOX. CHEM. NO.: 191

P.C. CODE: 018101

TEST MATERIAL (PURITY): CL(AC) 38,555 (Batch No. AC-6779-98A, 66.1% a.i.)

SYNONYMS: Chemically: (2-Chloroethyl) trimethylamonium chloride; chlormequat-chloride, (common name accepted by ISO); CYCOCEL®.

CITATION: Sharma, R.K. (1991). Evaluation of CYCOCEL® (CL 38,555) in the *In Vivo* Chromosome Assay in Rat Bone Marrow, performed at the Genetic Toxicology Laboratory of American Cyanamid, Princeton (NJ). Study No.: 90-14-001, dated 14 January 1991. MRID 41798101. Unpublished.

SPONSOR/SUBMITTER: American Cyanamid Company, Princeton (NJ).

EXECUTIVE SUMMARY:

In a mammalian cytogenetic assay (MRID 41798101), Sprague-Dawley rats (5M:5F per group) were administered the test material [CL(AC) 38,555, Batch AC 6779-98A, 66.1% a.i., in sterile water (SW)] at acute oral doses of 125, 250 and 500 mg/kg, and bone marrow cells were collected 12, 24 and 48 hours later. The characteristic array of chromosome aberrations in test groups was compared to that of the vehicle control rats. In addition to 5M and 5F administered the vehicle and sacrificed at the same harvest time as the test groups (to represent the vehicle, "negative" control), a group of 5M:5F was given a single administration of the mutagen, cyclophosphamide (CP, 40 mg/kg), and sacrificed at 24 hours. Two to three hours before harvesting, all cultures were administered colchicine, which arrests cell division at metaphase.

Deaths occurred at the highest test dose: One male from the 12-hour harvest, and 3 females from the 24-hour harvest. Other signs of toxicity included: Salivation; nose-bleeding; diarrhea.

103

There was no evidence (or a dose related positive response) of an increased number of abnormal metaphases of test substance-treated animals over background. The CP animals responded with marked increases in aberrations.

This study is classified as **acceptable/guideline** and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test Material: CL (AC) 38,555.

Description: Pale, yellow liquid.
Lot/Batch No.: AC 6779-98A (as provided in other studies of this series).
Purity: 66.1% a.i.
Vehicle: Sterile water (SW).
Stability of compound: The investigator stated that the test material was "stable", and referenced the following: "ARD Chemical Development Note Books AC 7163, pp. 33-38 and 55-58".

2. Control Materials:

Negative: None.
Vehicle/Final volume/route of administration: SW/10 mL/kg/oral gavage.
Positive/Final dose(s)/Route of administration: Cyclophosphamide monohydrate (CP, 40 mg/kg)/oral gavage.

3. Test Compound Administration:

Volume of test substance administered: 10 mL/kg.
Route of administration: Oral gavage.
Dose levels used:

a. Dose Range-Finding Test (3M:3F per dose): Single doses of 100, 200, 300, 400, 500 mg/kg.
b. Cytogenetic Assay (5M:5F per dose): Single doses of 125, 250, 500 mg/kg.

4. Test Animal:

a. Species: Rat Strain: Sprague-Dawley Age: 7 to 8 wks at dosing

Weight: Male: 210.9 to 238.4 g Female: 161.1 to 185.0 g

Source: (Not provided).

b. No. animals used per dose: 5 Males 5 Females.

c. Properly maintained: Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

a. Test compound and negative control:

Dosing: __x__ Once _____ Twice (24 hr apart)

_____Other (describe): None.

Sampling (after last dose): _____ 6 hr __x__ 12 hr

__x__ 24 hr __x__ 48 hr _____ 72 hr

b. Positive control:

Dosing: __x__ Once _____ Twice (24 hr apart)
Other (describe): None.
Sampling (after last dose): _____ 6 hr _____ 12 hr __x__ 24 hr
_____ 48 hr _____ 72 hr

c. Administration of spindle inhibitor:
Inhibitor used/dose: Colchicine/dose amount not provided
Route of administration: Intraperitoneal (i.p.)
Interval Administered before animal terminated: 2 to 3 hr

2. Tissues and Cells Examined:

__x__ bone marrow _____ other (list): None.
No. of cells examined per animal: 50
Other (if other cell types examined, describe): None.

3. Details of Slide Preparation:

Bone marrow cells were flushed into centrifuge tubes containing Hank's Balanced Salt Solution (HBSS). This cell suspension was centrifuged, cells swelled with hypotonic solution (0.075 M KCl), fixed by three changes of methanol: acetic acid (3:1), stained with 5% Giemsa, rinsed, and dropped onto glass slides. The slide preparations were air-dried. When dry, slides were dipped in xylene, and mounted under coverslips.

4. Evaluation Criteria:

A positive result was declared if metaphases on test slides showed statistically significant ($p \leq 0.05$) increased aberrations per cell compared to negative control, or increased percent aberrant cells by ANOVA analysis.

5. Statistical Methods:

Data from males and females were analyzed separately by ANOVA ("percent aberrant cells", as well as "aberrations per cell"). If necessary to stabilize variance, an arcsine transformation was performed. Also, data from each dose group were compared to the vehicle control using the "LSD" procedure for the number of aberrations per cell, and the percent abnormal cells, with significance set at the $P \leq 0.05$ probability level.

II. REPORTED RESULTS:

A. **Analysis**:

Five water solutions of Batch No. AC6779-98A from TGAI CL(AC) 38,555 for the range-finding phase of 100 to 500 mg/kg (10 to 50 mg/mL) assayed from 104% to 114% of nominals, with a mean of 108% and a coefficient of variation (CV) of 4%.

Three water solutions of Batch AC 6779-98A from TGAI CL(AC) 38,555 for the main (cytogenetics) assay at 125, 250 and 500 mg/kg (12.5, 25 and 50 mg/mL) assayed from 109% to 114% of nominals, with a mean of 112% and a CV of 2% (MRID 41798101, pp. 24 to 34).

B. **Dose Range-Finding Test**:

No deaths were observed at any dose. Toxic signs (salivating) were observed in all females at all doses, and in males gavaged with 400 and 500 mg/kg. Mitotic indices (MIs) were decreased at the highest dose tested (HDT), 500 mg/kg (MI = no. of dividing cells per 1000 cells counted). Based on these findings, 500 mg/kg was selected as the highest dose for the cytogenetic assay.

C. **Cytogenetics Assay (Chromosome Aberrations)**:

In the definitive cytogenetics assay, clinical adverse toxic signs (salivation, nose-bleeding, diarrhea, diuresis, chromodachryorrhea) were exhibited by "many" animals

[number affected not provided] at the high dose in all sacrifice groups, either immediately post-dosing or at colchicine administration. One 500 mg/kg male at the 12-hr harvest and three females at the 24-hr harvest died.

Data on chromosomal aberrations in metaphases revealed no significant effect of the test article in treated cultures compared to the vehicle control. Cyclophosphamide induced the expected significant ($p < 0.05$) increase in chromosome aberrations (MRID 41798101, pp. 13 to 24 - ATTACHMENT Tables 2 to 13).

Therefore, the investigator concluded that the test article was negative for causing chromosome damage in bone marrow cells of male and female Sprague-Dawley rats dosed up to clinical and cytotoxic levels.

III. REVIEWERS' DISCUSSION/CONCLUSIONS:

A. The EPA reviewers agree with the investigator's conclusions that the technical grade of CL(AC) 38,555 (chlormequat-chloride, CYCOCEL) was negative for inducing chromosome aberrations in Sprague-Dawley rats gavaged at doses up to clinical and cytotoxic levels.

B. Deficiencies: None.

CHLORMEQUAT-CHLORIDE

UNSCHEDULED DNA SYNTHESIS [84-2]

EPA Reviewer: Irving Mauer, Ph.D.
Registraton Action Branch 3, HED (7509C)
Secondary Reviewer: Nancy McCarroll
Toxicology Branch, HED (7509C)
TXR No.: 0054020.

Date: 07/19/06

Date: 07/19/06

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes/Mammalian Cell Cultures; OPPTS 870.5550 [84-2]; OECD 482.

DP BAR CODE: D325193 TOX. CHEM. NO.: 191

P. C. CODE: 018101

TEST MATERIAL (PURITY): AC 38,555 (Batch No. AC 6779-98A, 66.1% a.i.).

SYNONYMS: Chemically: (2-Chloroethyl)trimethyl ammonium chloride; chloromequat-chloride (common name suggested to ISO); CYCOCEL, trade name of American Cyanamid.

CITATION : Pant, K.J. (1990). Test for Chemical Induction of Unscheduled DNA Synthesis in Rat Primary Hepatocyte Cultures by Autoradiography with AC 38,555, performed at The SITEK Research Laboratories, Rockville (MD). SITEK Study No.: 0150-5100; dated November 12, 1990 (American Cyanamid Protocol: 971-90-148). MRID 41798103. Unpublished.

SPONSOR/SUBMITTER: American Cyanamid Company, Princeton (NJ).

EXECUTIVE SUMMARY:

In an unscheduled DNA synthesis (UDS) assay (MRID 41798103), cultures of primary rat hepatocytes were exposed to AC 38,555 (Batch No. AC 6779-98A, 66.1% a.i., dissolved in deionized, distilled water, DDW) at six concentrations ranging from 0.63 to 7.5 ul/mL plus 10 uCi/mL ^{3}H-thymidine (specific activity, 20 Ci/mM) for 18 hrs. Following an autoradiographic procedure, the cells were scored for silver grain counts, an indirect measure of UDS.

In a preliminary range-finding test, exposure to the test article for 18 hrs at 10 concentration levels of 0.08 to 100 uL/mL, both the average number of viable cells in replicate cultures, and the Relative Cell Survival (RCS) of treated compared to vehicle control groups, were calculated.

10

Based on the results indicating that concentrations of ≥50 uL/mL were lethal and the RCS was 8.3% at 10 and 41.7% at 5.0 ul/mL, 7.5 uL/mL was selected as the highest concentration for the main (definitive) UDS assay.

There was no evidence (or a dose-related positive response) that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced.

This study is classified as **acceptable/guideline** and satisfied the requirement for FIFRA Test Guideline 84-2 for other genotoxic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test Material: AC 38,555.
Description: Colorless to pale yellow liquid.
Lot/Batch No.: AC 6779-98A.
Purity: 66.1% a.i.
Stability of compound: Provided as an APPENDIX, pp. 34 to 40 of MRID 41798103.
CAS No.: 999-8-5 (From other studies in this series).
Vehicle/Solvent used: Deionized, distilled (DDW).
Other comments: Stored at room temperature.

2. Control Materials:

Negative: None.
Solvent/Final concentration: DDW/Not provided.
Positive (concentrations, solvent): 2-Acetylaminofluorene (2AAF, 10 *u*g/mL, dissolved in ethanol).

3. Test Compound Concentrations Used:

a. Preliminary Range-Finder: 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5.0, 10, 50, 100 *u*l/mL (in duplicate per concentration).

b. Main Assay: 0.63, 1.25, 2.5, 4.0, 5.0, 7.5 *u*l/mL (in triplicate per concentration).

4. Media:

Williams Medium E (WME), buffered with 0.01 M HEPES, and supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 IU/mL penicillin and 100 *u*g/mL streptomycin.

5. Test Cells:

Mammalian cells in culture/primary rat hepatocytes from male Sprague-Dawley rats weighing 200 to 325 g, purchased from Charles River Laboratories, Raleigh [NC]. Test cells were obtained by *in situ* collagenase perfusion of the liver.

6. Cell Preparation:

Perfusion Technique and Hepatocyte Harvest/Culture Preparation:

Livers were perfused and hepatocytes were isolated according to the procedures of Williams[1, 2], and Bradlaw[3].

B. TEST PERFORMANCE:

1. Treatment:

Prepared hepatocyte cultures were exposed for 18 hours to the test article and 10 μCi/mL ^{3}H-thymidine (specific activity, 20 Ci/mM).

After exposure, the cultures were repeatedly washed in WME (above), the cells were swollen in 1.0% sodium citrate, and fixed in several changes of methanol:acetic acid (3:1). Cells mounted on coverslips were affixed (cell side out) onto standard microscope slides, which were dipped into photographic NTB-2 emulsion at 43-45°C, and allowed to drain at room temperature in the dark for 90 minutes. Following this, slides were stored at 0-4°C in light-tight slide boxes containing a dessicant for 6 days. After this exposure, slides were developed in Kodak D-19, followed by Kodak's fixer, stained in hematoxylin, mounted in Permount and covered with thin coverslips.

Slides were coded and read "blind." Silver grains of 150 randomly selected nuclei (each accompanied by three background, *i.e.*, cytoplasmic, counts) were counted per dose level, employing an electronic colony counter equipped with a microscope-mounted auxiliary television camera. Only morphologically normal-appearing cells were scored.

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[1]Williams, G.M. Carcinogen-induced DNA repair in primary rat liver cell cultures, a possible screen for chemical carcinogens. Canc. Lett., *1*:231-237, 1977.

[2]Williams, G.M. The detection of chemical mutagens/carcinogens by DNA repair and mutagenesis in liver cultures. In: Chemical Mutagens, Vol VI, F.J. DeSerres and A. Hollander, Eds. Plenum Press, N.Y., pp. 71-79, 1979.

[3]Bradlaw, J. Food and Drug Administration, Washington, D.C., Personal Communication.

Incorporation of radioactive thymidine into nuclear DNA was determined by counting the darkened grains therein, and the net nuclear grain counts (NNGC) were recorded by subtracting the average cytoplasmic counts. The numbers of nuclei showing five or more NNGC were also recorded.

In addition, 300 cells per culture exhibiting heavily darkened nuclei, containing grains too numerous to count (TNTC) were scored to determine nuclei undergoing scheduled (S)-phase DNA synthesis.

2. Criteria:

Criteria for valid assays and evaluation of test results were both presented. Responses were recorded as:

a. Positive: Dose-related, with one dose exhibiting a significant increase over concurrent negative control; **or** in the absence of a dose-related response, at least two successive doses exhibited significant increases over control.

b. Marginally positive: Only one test dose showing a significant response.

c. Negative: No indication of a positive at any test dose.

II. REPORTED RESULTS:

A. **Chemical Analysis**:

Seven water solutions for the Range-Finding Test in the range of 8 to 500 uL/mL assayed from 100% to 124% of nominal, with a mean of 113% and a coefficient of variation (CV) of 7%.

Six water solutions for the main (UDS) assay in the range of 62.5 to 750 uL/mL assayed from 99% to 108% of nominal, with a mean of 103% and a CV of 4% (MRID 41798103, pp. 34 to 36).

B. **Preliminary Range-Finding Test**:

Concentrations of 50 and 100 uL/mL were lethal. Based on these results, RCS ranged from ≥ 86% at levels ≤ 2.5 uL to 8.3, or 41.7% at 10 or 5 uL/mL,

respectively. Based on these results, concentrations ranging from 0.65 to 7.5 *u*L/mL for the main (UDS) assay were selected (MRID 41798103, p. 16 -ATTACHMENT Table 1).

C. **UDS Assay**:

The top four doses (2.5, 4.0, 5.0 and 7.5 *u*L/mL) plus controls were scored for UDS. None of these concentrations showed a significant increase in average NNGC over the concurrent vehicle control. The positive control, 2AAF, induced a marked increase in UDS (MRID 41798103, p. 17 -ATTACHMENT Table 2).

Therefore the investigator concluded that AC 38,555 did not produce a significant increase in NNGC over control value at concentrations approaching severe cytotoxicity.

III. REVIEWER'S CONCLUSION:

A. The EPA reviewers agree with the investigator that AC 38,555 (chlormequat-chloride, CYCOCEL) is not genotoxic in inducing UDS in primary rat hepatocytes up to sublethal concentrations (≤7.5 *u*L/mL).

B. **Deficiencies**: None.